INTERACTIONS BETWEEN PARTIAL REPLACEMENT OF BARLEY GRAIN WITH DRIED WHEY PERMEATE AND DIETARY RUMINALLY-DEGRADABLE PROTEIN LEVEL ON RUMINAL FERMENTATION CHARACTERISTICS, NITROGEN UTILIZATION, UREA-N RECYCLING, AND PRODUCTION PERFORMANCE IN DAIRY COWS

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ABSTRACT

The objective of this experiment was to delineate the interactions between partial replacement of barley grain with dried whey permeate (DWP; a source of lactose) and dietary rumen-degradable protein levels on feed intake, milk production, ruminal fermentation characteristics (ruminal pH, and SCFA and ammonia-N concentrations), whole-body urea-N kinetics, nitrogen balance, and total-tract nutrient digestibility. Eight lactating Holstein cows (738 $\pm$ 98 kg BW; 93 $\pm$ 39 DIM at the beginning of the experiment) were used in a replicated 4 x 4 Latin square design experiment with a 2 x 2 factorial arrangement of dietary treatments and 28-d periods (18 d of dietary adaptation and 10 d of data and sample collection). One square had four ruminally-cannulated cows for measurement of ruminal fermentation characteristics, whole-body urea-N kinetics, nitrogen balance, and total-tract nutrient digestibility. The dietary treatment factors were: 1) two levels of dietary inclusion of DWP (0 vs. 11.2%; dry matter [DM] basis): and 2) two levels of dietary RDP (9.5% vs. 11.5%; expressed as % of DM). Dietary RDP level was manipulated by inclusion of untreated or heat-treated soybean meal. Dietary addition of DWP increased water-soluble carbohydrate (WSC) content from 4.8% (range = 4.61 to 5.04%) in the diet without added DWP to 10.7% (range = 10.4 to 11.0%) in the diet with added DWP. The dietary addition of DWP tended to increase DMI ($P = 0.09$) but did not affect milk yield, or fat content and yield ($P > 0.05$), while milk protein content ($P = 0.01$) and yield increased ($P = 0.01$). Milk lactose content was similar in cows fed 9.5 and 11.5% RDP without added DWP, but milk lactose content was greater in cows fed 9.5% RDP compared to those fed 11.5% RDP with added DWP ($P < 0.01$), but dietary treatment had no effect on milk lactose yield ($P \geq 0.53$). Dietary treatments did not affect mean ruminal pH, minimum daily pH, and area or duration ($P > 0.05$) when ruminal pH was less than 5.8. Cows fed the low RDP diet had a greater maximum ruminal pH compared those fed the high RDP diet ($P = 0.03$). Dietary treatments did not affect ruminal concentrations of acetate, propionate, and ammonia-N ($P > 0.05$), whereas the dietary inclusion of DWP decreased ruminal concentrations of isobutyrate, isovalerate, total BCFA, and total SCFA ($P < 0.05$). Increasing dietary RDP content increased the ruminal concentration of isovalerate ($P = 0.03$) and tended to increase ruminal concentrations of valerate and total BCFA ($P = 0.08$), while rumen concentration of total SCFA increased ($P < 0.01$). Dietary addition of DWP tended to increase the total-tract digestibility of organic matter (OM), water-soluble carbohydrates (WSC), and ether extract (EE) ($P < 0.05$). Total-tract crude protein (CP) digestibility was higher in cows fed 11.5% RDP

compared to those fed 9.5% RDP ($P = 0.02$). Dietary addition of DWP tended to increase N intake over the period when N balance was measured ($P = 0.08$), and increased urinary N and fecal N excretion (g/d) , and milk N secretion (g/d) ($P < 0.05$) without affecting total N excretion (expressed as g/d and % of N intake) ($P > 0.05$). Increasing dietary content of RDP tended to increase N intake ($P = 0.08$) but decreased fecal N excretion (% of N intake), total N excretion (% of N intake) ($P < 0.05$), and tended to decrease milk N secretion (% of N intake) ($P = 0.08$). Apparent nitrogen balance was similar in cows fed 9.5 and 11.5% RDP with added DWP, but apparent nitrogen balance was higher in cows fed 11.5% RDP compared to those fed 9.5% RDP without added DWP ($P = 0.02$). A similar interaction effect was observed for productive N ($P = 0.04$). The dietary addition of DWP increased urea-N entry rate (UER) ($P < 0.01$). There were no diet effects ($P > 0.05$) on urea-N returned to the ornithine cycle (ROC), urinary urea-N excretion (UUE), urea-N utilized for anabolism (UUA), and all fractional urea-N transfers. Increased dietary RDP content decreased UER ($P = 0.03$), and urea-N gut entry rate (GER) tended to be higher in cows fed the low RDP diet compared to those fed the high RDP diet ($P = 0.10$), and the low RDP diet had a higher urea-N loss to feces compared to the high RDP ($P = 0.02$). The results show that the partial substitution of barley grain with DWP does not increase the susceptibility of cows to ruminal acidosis and has no impact on animal productivity. Increasing dietary RDP content reduced overall N excretion; however, the dietary RDP changes in this experiment were likely not large enough to stimulate changes in animal productivity or change urea-N recycling.

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[15N15N]-urea, double-labelled urea

AA, amino acids

ADF, acid detergent fibre

AOAC, association of official analytical chemists

AQP, aquaporin

BCFA, branched-chain fatty acid

BHBA, beta-hydroxybutyrate

BUN, blood urea-nitrogen

BW, body weight

CP, crude protein

DIM, days in milk

DM, dry matter

DMI, dry matter intake

DWP, dried whey permeate

ECM, energy-corrected milk

EE, ether extract

FCM, fat-corrected milk

GC, gas chromatography

GER, GIT entry rate (urea-N transfer to the GIT)

IGF, insulin-like growth factor

MP, metabolizable protein

MPS, microbial protein synthesis

MUN, milk urea nitrogen

N, nitrogen

NDF, neutral detergent fibre

NEL, net energy of lactation

NFC, non-fibre carbohydrate

NH3/NH4+, ammonia,

NPN, non-protein N

NRC, National Research Council

NSC, non-structural carbohydrate

OM, organic matter

PUN, plasma urea nitrogen

RDP, ruminally-degradable protein

RFC, readily-fermentable carbohydrate ROC,

ROC, urea-N return to the ornithine cycle

RUP, ruminally-undegradable protein

SARA, sub-acute ruminal acidosis

SCFA, short-chain fatty acid

TMR, total mixed ration

TS, total sugar

UER, urea-N entry rate (endogenous production of urea)

UFE, urea-N loss to feces UUA,

UT-A/B, urea transporter protein

UUA, urea-N utilized for anabolism UUE,

UUE, urinary urea-N excretion

VFA, volatile fatty acids

WSC, water-soluble carbohydrate

1   GENERAL INTRODUCTION

High-producing dairy cow diets are formulated to be highly fermentable to provide adequate energy to support maintenance, reproduction, and lactation (Iqbal et al., 2012). The main source of energy for cows is carbohydrates, contributing an average of 70% of dry matter intake (DMI) (Oba, 2011). Carbohydrates are fermented into volatile fatty acids (VFA) in the rumen, which are then absorbed by the ruminal epithelia. Cereal grains are the main source of digestible carbohydrates in dairy diets (Oba, 2011). In western Canada, the main cereal grain used in dairy diets is barley because of its high local availability (Iqbal et al., 2012). Compared to other cereal grains like corn, barley is fermented at a high rate which leads to rapid production of VFA in the rumen (Offner et al., 2003; Iqbal et al., 2012). High VFA production is desirable in ruminants because it means there is more metabolic energy available, but VFA production leads to the release of protons which, in turn, may lower the pH in the rumen if the rate of VFA production is not matched by their neutralization and absorption (Yang et al., 1997; Iqbal et al., 2012). If the rate of VFA removal from the rumen is low or if there is insufficient buffering, the drop in pH may cause ruminal acidosis (Beauchemin et al., 2006; Krause and Oetzel, 2006). Because of barley's high fermentation rates and high starch content, animals fed on barley-based diets are at a higher risk of ruminal acidosis compared to those fed other cereal grains.

Ruminal acidosis is a nutritional disorder which is characterized by rapid accumulation of acids in the rumen caused by an imbalance between fermentation acid production and acid neutralization and acid removal (Krause and Oetzel, 2006). Ruminal acidosis is triggered by either insufficient buffering caused by low dietary fibre, rapid intake of highly fermentable carbohydrates or inadequate ruminal adaptation to high fermentation rates (Krause and Oetzel, 2006). There are two main forms of ruminal acidosis, which are acute ruminal acidosis characterized by pH below 5.2, and sub-acute ruminal acidosis (SARA) which has pH ranges of between 5.8 and 5.2 (Penner et al., 2007). Clinical signs of ruminal acidosis include anorexia, elevated temperature, diarrhea, lethargy, tachypnea, recumbence and even death, but final diagnosis is usually by measuring ruminal pH (Krause and Oetzel, 2006). Ruminal acidosis reduces productivity and is associated with laminitis (Nocek, 1997), reduced feed intake, reduced milk fat content (Kleen et al., 2003) and liver abscesses (Nagaraja and Lechtenberg, 2007). Ruminal acidosis also affects animal

welfare because of its association with lameness, which is one of the main causes of early culling in dairy herds (Krause and Oetzel, 2006).

Ruminal acidosis is a major problem in beef and dairy production around the world with research in the US by Gao and Oba (2014) showing incidence rates of SARA between 19% and 26% during lactation. Studies on feedlot steers in Canada have shown ruminal acidosis rates of up to 37.8% (Penner, 2014). Because of these high prevalence rates, ruminal acidosis causes significant financial losses to dairy farms. Ruminal acidosis causes losses of approximately US$475 per cow per year because of a decrease in milk production of 3 kg per cow per day and decreases of milk fat and true protein content by 8.1% and 3.44%, respectively (Krause and Oetzel, 2006).

Ruminal acidosis can be managed by either reducing the quantity of starch consumed by the cows, reducing feed intake or increasing the forage to concentrate ratio which, in turn, would increase saliva production which contains buffers that neutralize VFA in the rumen (Owens et al., 1998). However, these strategies can reduce VFA production in the rumen. Because VFA are the major source of energy for maintenance and productive functions in dairy cows, a reduction in ruminal VFA production would limit animal productivity. Another potential strategy to reduce ruminal acidosis without affecting productivity is the substitution of starch with sugar. Sugars are a class of carbohydrates that are water-soluble which includes the monosaccharides fructose, galactose and glucose, and the disaccharides lactose, maltose and sucrose (Oba, 2011). Sugars are known to ferment faster than starch in the rumen with rates above 500% per hour (Weisbjerg et al., 1998). However, numerous studies have shown that when starch is substituted with sugar there is no significant change in ruminal pH despite the increased fermentation rates (Defrain et al., 2004; Vallimont et al., 2004). In fact, a study by Penner et al. (2009) showed an increase in ruminal pH with increasing sucrose content in the diet as a substitute for cracked corn grain. Gäbel et al. (1991) suggested that changes in proportions of VFA production when diets increase fermentability may increase the permeability of ruminal epithelia. Sugar inclusion has been shown to increase the rate of butyrate production in the rumen (DeFrain et al., 2006). When butyrate is absorbed into the ruminal wall, most of it is metabolized into β-hydroxybutyrate (BHBA) to provide energy for ruminal epithelia (Weigand et al., 1975). Increasing butyrate metabolism is known to stimulate growth and proliferation of ruminal epithelia (Tan and Murphy, 2004) through

the effects of IGF-1 and EGF (Penner et al., 2011). Increases in ruminal epithelia cell surface area can help explain why pH does not decrease on high sugar diets. There is also experimental evidence that shows an increase in the expression of ruminal epithelial transport proteins. Chibisa et al. (2015) reported that when DWP partially replaces corn and barley grain, there was an increase in Cl^--competitive absorption rates of propionate and acetate. Cl^--competitive absorption also increases HCO_3^- secretion into the rumen, which could potentially increase ruminal buffering capacity (Chibisa et al., 2015).

Partial replacement of starch with sugar improves DMI, fat-corrected milk (FCM) yield, milk protein and fat contents, and feed digestibility (Broderick and Radloff, 2004; Defrain et al., 2004; Vallimont et al., 2004; Broderick et al., 2008). By-products that have high sugar content and can be fed to ruminants include whey products, molasses, citrus pulp and corn steep liquor (Oba, 2011). Whey permeate contains approximately 70% lactose on a dry matter basis (Oba, 2011), so it is a rich source of sugar. In Saskatchewan, liquid whey is readily available locally from the Saputo milk processing plant in Saskatoon. Research by Defrain et al. (2004) showed that the replacement of cornstarch with liquid whey did not affect ruminal pH, but it decreased milk urea nitrogen (MUN) concentration and increased DMI. Steers have been fed diets with up to 60% liquid whey on a dry matter basis without affecting digestion and growth (King and Schingoethe, 1983; Susmel et al., 1995).

The nitrogen (N) that is contained in dairy diets as true protein or NPN (through microbial protein synthesis) is important because it supplies amino acids that are the precursors for milk protein synthesis (Arriola Apelo et al., 2014). However, the efficiency of utilization of dietary N is very low with only 20 to 35% of dietary N being incorporated into milk N (Arriola Apelo et al., 2014), and up to 80% of dietary N can be lost in urine and feces (Mulligan et al., 2004). Nitrogen excretion is a concern because of its environmental impact, and it has been linked to eutrophication, global warming, costal hypoxia, and acidification of rain, soil and surface water (Arriola Apelo et al., 2014). Animal farming has been estimated to contribute an average of 9% of all greenhouse emissions (O'Mara, 2011). Nitrogen loss also has impacts on human health because it aggravates asthma and pollutes drinking water (Arriola Apelo et al., 2014). Nitrogen is mainly lost from the animal in the form of ammonia, urea in urine and fecal N.

The efficiency of conversion of feed N into milk crude protein is poor for a number of reasons, including the lack of synchrony between energy availability (from carbohydrate fermentation) and N availability (from crude protein fermentation) for efficient microbial protein synthesis (Tan and Murphy, 2004). This is particularly so because dairy cows are fed high forage diets that contain fermented forages (silages) which are high in ruminally-degradable protein (RDP). When the RDP is fermented in the rumen without a matching supply of ruminally-fermentable carbohydrate, the asynchronous availability of N and energy results in increased rumen ammonia concentrations, increased ammonia absorption into the blood, and conversion into urea by the liver and excretion in urine (McCormick et al., 2001). Urinary N excretion represents an irreversible loss of N for the dairy cow. Increasing carbohydrate fermentation has been shown to increase synchrony, thereby leading to more efficient utilization of RDP by ruminal microorganisms (Broderick and Radloff, 2004).

Research on sugar has shown that sugar is more fermentable than starch (Oba, 2011), which is traditionally used as an energy source in dairy and beef cattle diets and substitution of starch in diets by sugar may improve RDP utilization. This is supported by the Cornell Net Carbohydrate and Protein System (NRC, 2000) shows that ruminal microbes that utilize sugar produce 18% more microbial protein than microbes that utilize starch (Broderick and Radloff, 2004). Past research has demonstrated that the inclusion of sugars in diets can improve utilization of feed N content. Many studies have shown that dietary inclusion of sucrose decreases the concentration of ammonia in ruminal fluid (Chamberlain et al., 1993; Sannes et al., 2002; Broderick et al., 2008). In a study to investigate the effects of sugar supplementation in the form of molasses on the productivity of dairy cows, substitution of starch with molasses reduced concentrations of ammonia in the rumen and excretion of N in the urine (Broderick and Radloff, 2004). Ammonia concentration in the rumen is related to the efficiency of N utilization. In another study, Holstein cows were fed sucrose-fortified grain, and this lowered ruminal ammonia concentration (McCormick et al., 2001).

This thesis research investigated the interactions between partial replacement of barley grain with DWP and dietary ruminally-degradable protein level on ruminal fermentation characteristics, nitrogen utilization, urea-N recycling, and production performance in dairy cows.

2 LITERATURE REVIEW

2.1 Sources of energy in dairy cow diets

Over the past half century, dairy cow productivity has more than doubled, with an average dairy cow now producing 145% more milk than in 1973 (Dairy Farmers of Canada, 2017). Increases in dairy cow productivity are due to improvements in nutrition, animal management, animal welfare, and improvements in genetic selection through the application of principles of genetics (Oltenacu and Broom, 2010). The modern dairy cow's improved productivity has increased energetic demands and, to provide sufficient energy to support maintenance, reproduction, and lactation, diets are formulated to be highly fermentable (Iqbal et al., 2012). Diets that have high fermentation rates have been shown to increase productivity compared to less fermentable diets (Oba and Allen, 2003). The main source of energy for cows is carbohydrates, contributing an average of 70% of DMI (Oba, 2011). Carbohydrates are fermented into volatile fatty acids (VFA) in the rumen, which are then absorbed by the ruminal epithelia and used to meet the energy needs of cows. Cereal grains are the main source of carbohydrates in dairy diets (Oba, 2011). The most common cereal grains used in dairy feed formulation are corn, barley, oats, and wheat. In western Canada, the main cereal grain used in dairy diets is barley because of its higher local availability (Iqbal et al., 2012). Corn is also gaining popularity because of the development of varieties that can grow with less heat units (Lardner et al., 2012). Compared to other cereal grains, barley is fermented at a high rate, which leads to rapid production of VFA in the rumen (Offner et al., 2003; Iqbal et al., 2012).

Of equal importance to energy supply to ruminants are simple sugars, which contribute a significant part of feedstuff like molasses, citrus products, whey, and fresh fodder (Weisbjerg et al., 1998; Penner, 2015). Sucrose is the most common sugar present in livestock feed, other simple sugars that may be present in feed depending on ingredients are lactose, maltose, glucose and fructose (Hall, 2002; Zadeh and Kor, 2013). Simple sugars like lactose can be added to diets up to 12.5% of dietary DM without negatively affecting productivity (De Seram, 2016). Simple sugars have faster fermentation rates compared to starch and other structural carbohydrates (Van Amburgh et al., 2015), suggesting that they can support greater productivity than other carbohydrate fractions.

5

2.2   Digestion of starch

Starch digestion mainly occurs in the rumen, and to a lesser extent, in the small intestines (Ferraretto et al., 2013; Owens, 2015). Ruminal starch degradation is accomplished by several amylolytic bacteria species acting symbiotically, and to a lesser extent, protozoa and fungi (Huntington, 1997). Ruminal microbes ferment carbohydrates into VFA, which are the major source of energy for ruminants (Giuberti et al., 2014). Ruminal starch degradation ranges from 25-100% of dietary starch intake (Swingle et al., 1999), and the rate and extent of ruminal starch degradation is influenced by type of grain, passage rate, mechanical and chemical processing of the grain, DMI and ruminal bacteria adaptation to the diet (Huntington, 1997).

The first stage in ruminal starch degradation is microbial attachment to starch particles (Giuberti et al., 2014). Particle-associated bacteria are responsible for close to 75% of starch digestion in the rumen (McAllister et al., 1994). Particle-associated bacteria produce extracellular enzymes that are found on their cell walls (Kotarski, S. F., 1992). Bacterial endo- and exo-enzymes breakdown α1-4 glycosidic bonds present in amylose, and α1-6 and α1-4 glycosidic bonds present in amylopectin (Huntington, 1997). The α-amylase and β-amylase produced by ruminal microbes cleave glycosidic bonds from both amylose and amylopectin to produce glucose, maltotriose, maltose and α- limit dextrin. To finish hydrolysis to glucose, bacteria secrete glucoamylase and maltase to produce the end-product, glucose. Glucose is then absorbed by microbial cells and fermented into VFA.

Some starch may escape complete ruminal degradation and is further digested in the small intestine. When starch reaches the small intestine, it is hydrolyzed into dextrin and oligosaccharides by pancreatic α-amylase, and finally into glucose by maltase and isomaltose found on intestinal villi (Huntington, 1997). Glucose is absorbed into portal circulation using sodium-glucose transporter proteins. Between 5-20% of dietary starch escapes ruminal degradation and intestinal digestion (Huntington, 1997; Ferraretto et al., 2013; Owens, 2015), and up to 95% of starch can be digested over the total digestive tract (Tucker et al., 1968). Simple sugars on the other hand have higher degradation rates compared to starch (40-60 vs 20-40 Kd, %/h) (Van Amburgh et al., 2015), and are quickly fermented into VFA by ruminal microbes (Zadeh and Kor, 2013).

2.2.1   Microbes involved with starch degradation

Amylolytic bacteria are responsible for the bulk of ruminal degradation of starch, and constitute up to 95% of culturable rumen bacteria in a grain-fed animal (Leedle et al., 1982). Some of the major bacterial species that are involved in ruminal starch degradation that have been isolated so far include *Streptococcus bovis, Succinimonas lactilytica, Bacteroides amylophilus, Lactobacillus spp, Butyrivibrio fibrisolvens, Eubacterium ruminantium, Prevotella ruminocola, Bacteroides ruminocola,* and *Ruminobacter amylophilus* (Kotarski, S. F., 1992). Ruminal bacteria have extracellular amylolytic enzymes, including beta-amylase, alpha-amylase, r-amylase, and iso-amylase, which help to digest starch into glucose by breaking the bonds present in starch (Cerrilla and Martínez, 2003). Not all bacteria possess the complete set of enzymes that are capable of digesting starch into its monomers, so a wide array of bacteria act synergistically to digest starch (Cotta, 1992).

Ciliated protozoa are also involved with starch degradation. *Entodinium* genus is the most common protozoa found in ruminants fed high grain diets because of the protozoa's resistance to low pH that usually characterizes high grain feeding (Nagaraja and Titgemeyer, 2007). Ruminal protozoa are known to reduce rates of starch degradation and VFA production because they sequester starch and simple sugars. Protozoa engulf and ingest starch granules, which they convert to storage carbohydrates and slowly digest (Mendoza et al., 1993; Nagaraja and Titgemeyer, 2007). Protozoa impact carbohydrate digestion by engulfing starch granules, thus making them unavailable to bacteria which, in turn, slows down ruminal starch digestion. In experiments involving sheep, ruminal starch degradation was improved by defaunation (Mendoza et al., 1993).

2.2.2   Volatile fatty acid production in the rumen

The rumen is an anaerobic environment, so ruminal microbes cannot completely degrade carbohydrates into water and CO_2; instead, microbes obtain their ATP from the fermentation of carbohydrates with the production of VFA as the major by-product. Acetate, propionate and butyrate are the major VFA produced, and these have major energetic value to the ruminant animal supplying up to 80% of the ruminant's total energetic requirements (Bergman et al., 1965). Extracellular digestion of starch results in the release of glucose, which is then transported into microbial cells and goes through the glycolytic pathway to produce pyruvate. Pyruvate is fermented into volatile fatty acids by ruminal microbes.

Ruminal acetate synthesis involves two main pathways (Figure 2.1). The first one involves the conversion of pyruvate into acetyl-CoA with the production of CO_2 and H_2 as by-products, acetyl-CoA is then converted into acetate. The second pathway involves pyruvate being cleaved into formate and acetyl-phosphate, then acetyl-phosphate is converted to acetate (Van Houtert, 1993).

Production of propionate also has two pathways (Figure 2.1), the randomizing pathway and non-randomising pathway. The non-randomising pathway is common in animals fed high concentrate diets which favour lactate production, where propionate is produced via conversion of pyruvate to lactate and acrylate, and lactate is then converted into propionate. In the randomizing pathway, oxaloacetate is formed from pyruvate and CO_2. Oxaloacetate then undergoes a series of carboxylation reactions to form propionate. The randomizing pathway is more common in animals on a high fibre diet (Van Houtert, 1993).

Butyrate is produced via two pathways (Figure 2.1). The first one involves the conversion of two moles of acetate into one mole of butyrate. Two molecules of acetate are converted into acetyl-CoA, and the two molecules of acetyl-CoA are then converted to acetoacetyl-CoA which is then converted to butyrate via a series of reactions. The second pathway involves acetyl-CoA being converted to malonyl-CoA, which then reacts with another molecule of acetyl-CoA to form acetoacetyl-CoA (Van Houtert, 1993).

2.2.3 Volatile fatty acid absorption across the ruminal epithelium

For optimum ruminal function and health, it is important to maintain ruminal VFA concentration between 80 to 170 mM and ruminal pH between 5.6 to 6.5 (Nagaraja and Titgemeyer, 2007). This is achieved by removal or buffering of VFA. Most of the VFA produced in the rumen is removed via absorption, and a small portion of ruminal VFA is removed by passage out of the rumen; 9.7 %/h compared to absorption rates of 24.2 %/h in high concentrate-fed cows (Penner et al., 2009c). Fractional passage rates of VFA is influenced by DMI, and feed chemical and physical characteristics (Dijkstra et al., 2012). Energy intake has the largest effect on the ratio of VFA that is either absorbed or passed out of the rumen (Aschenbach et al., 2011), and fractional passage rates of VFA range from 20 to 40 % depending on the diet (Dijkstra et al., 2012).
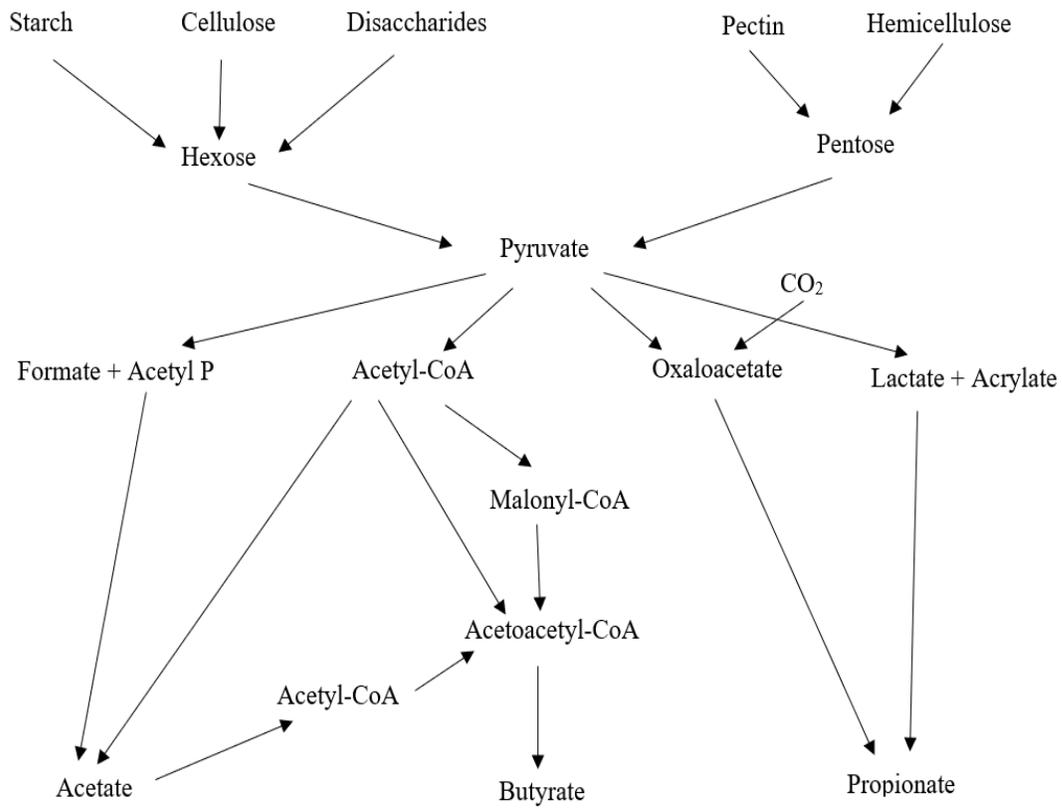
Figure 2.1 Pathways for fermentation of carbohydrates to VFA

There are three main mechanisms that are used to transport VFA across the rumen epithelia, namely passive diffusion, bicarbonate-independent and bicarbonate-dependent absorption (Aschenbach et al., 2009). Volatile fatty acid absorption ranges from 50 to 85% of total ruminal VFA concentration (Allen, 1997; Penner et al., 2009a).

The mechanism for absorption of volatile fatty acids

Initially, VFA absorption was attributed only to passive diffusion (Bugaut, 1987). Passive diffusion involves undissociated volatile fatty acids, which are lipophilic and can easily diffuse out of the rumen down a concentration gradient (Figure 2.2). Passive diffusion also leads to equal removal of protons from the rumen, which helps with ruminal pH regulation (Aschenbach et al., 2011). Passive diffusion is dependent on ruminal pH and VFA chain length. Longer chain VFA have a higher lipophilicity compared to VFA with shorter hydrocarbon chains, and their rate of diffusion is faster (Walter and Gutknecht, 1986). Volatile fatty acid's hydrocarbon chain length and rate of absorption is in the order butyrate > propionate > acetate (Walter and Gutknecht, 1986). The state of VFA (dissociated or undissociated) is dependent on their pKa. The pKa for VFA is pH 4.8 and according to the Henderson-Hasselbach equation at normal ruminal physiological conditions only a small proportion of VFA are in the undissociated form (Dijkstra et al., 1993; López et al., 2003), which means that the role of passive diffusion in the removal of VFA from the rumen and pH regulation is minimal. This shows that passive diffusion is not the main or only pathway for VFA removal from the rumen, particularly in animals fed high concentrate diets, which usually leads to lower ruminal pH as compared to high forage diets.

Dissociated VFA are removed using non-diffusional transportation pathways that require transport proteins (Aschenbach et al., 2011). The VFA/HCO_3 anion exchange pathway is the main non-diffusional pathway for absorption of dissociated VFA (Aschenbach et al., 2011). Studies done with sheep showed that the VFA/HCO_3 anion exchange pathway could account for up to 50% of VFA absorption (Penner et al., 2009a). Bicarbonate-dependent absorption is influenced by ruminal pH and ruminal VFA concentration, with absorption increasing with decreasing ruminal pH (Aschenbach et al., 2009). The bicarbonate secreted into the rumen plays a significant role in ruminal pH regulation (Aschenbach et al., 2011). Other documented pathways for VFA absorption include electrogenic VFA- transport (Stumpff et al., 2009) and the nitrate-sensitive transport pathway (Aschenbach et al., 2011); however, little is known about these two pathways.
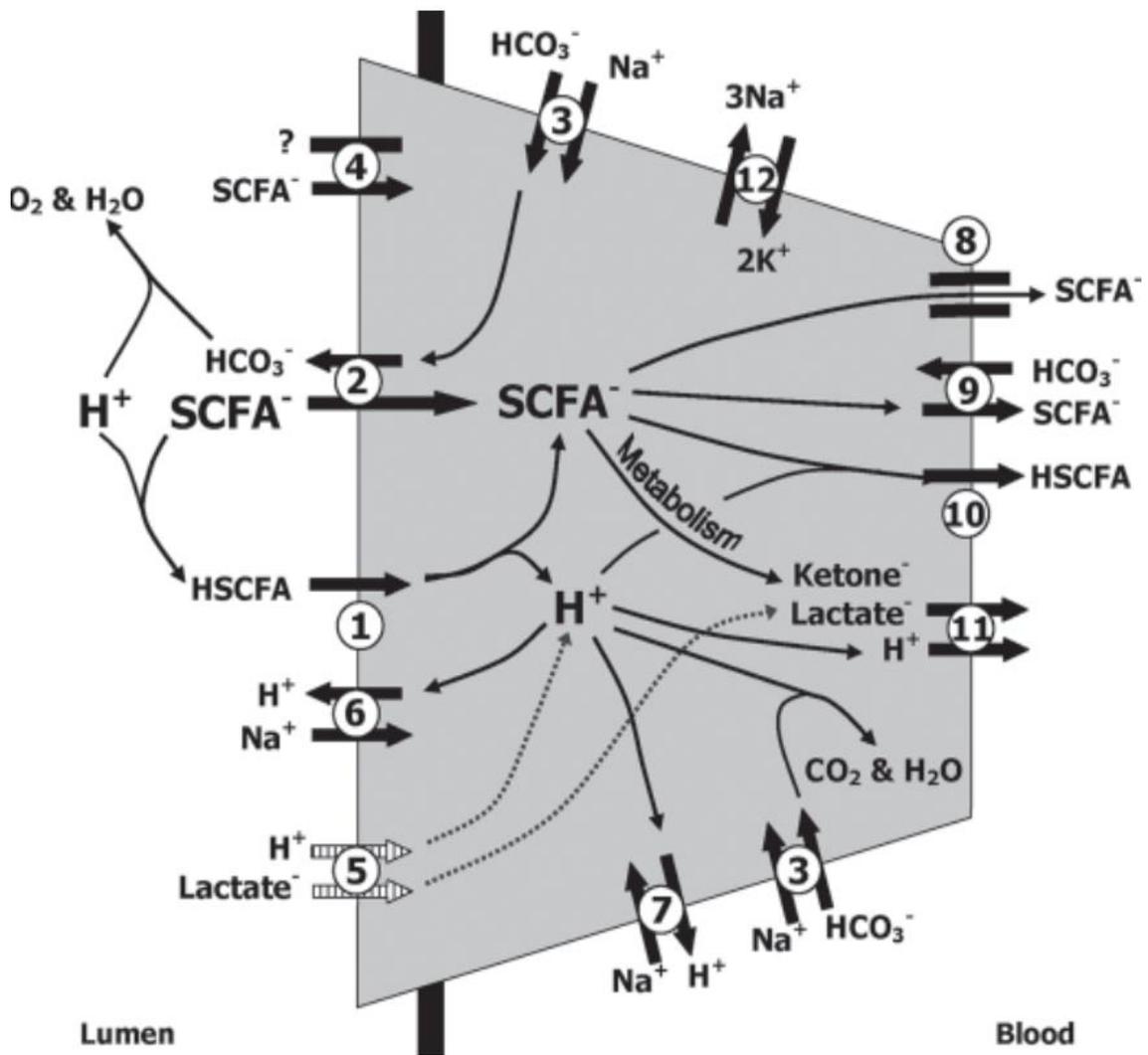
Figure 2.2 Organic acid transport across epithelial cells (Aschenbach et al., 2011)

2.2.3.1   Factors affecting absorption of volatile fatty acids

The rate of VFA removal from the rumen determines the animal's ability to cope with high rates of VFA production, and this is affected by many ruminal and dietary factors. Dietary fibre intake increases rates of volatile fatty acid absorption. Dietary fibre forms a mat in the rumen that stimulates ruminal motility and mixing, increasing the frequency of ruminal contractions (Beauchemin et al., 2006), brings VFA closer to the ruminal epithelia wall and increases VFA absorption (Hungate, 1966). Absorption of VFA is affected by diet forage to concentrate ratio. Penner et al. (2009) observed an increase in absorption rates of propionate and butyrate in cows fed a high concentrate diet compared to a low concentrate diet. This is probably due to increasing VFA concentrations in the rumen, which increases VFA absorption. Increasing dietary concentrate may also increase VFA absorption because of its relationship with ruminal pH. A washed reticulorumen technique experiment by Dijkstra et al. (1993) showed increased absorption of propionate and butyrate when ruminal pH was decreased from 7.2 to 4.5. Other factors that influence VFA absorption include ruminal volume (Dijkstra et al., 1993), ruminal osmolarity (Owens et al., 1998), and ruminal papillae surface area (Dirksen et al., 1985).

2.2.4   Volatile fatty acid utilization by dairy cows.

The ruminant body utilizes VFA absorbed from the rumen, and plasma concentrations of VFA are significantly lower than the quantity of VFA that are absorbed from the rumen, (Van Houtert, 1993). The rumen epithelia metabolize a significant portion of the VFA during absorption, and it receives some of its energetic requirements from them. Up to 12% of the propionate absorbed from the rumen is oxidized into CO_2 or converted to glucose via lactate by ruminal epithelial cells (Bergman and Wolfe 1971; Kristensen, 2005). Butyrate is also metabolized by the ruminal epithelia tissue, with 90% of the butyrate produced by the rumen being converted to beta-hydroxybutyrate (BHBA) by the ruminal epithelia tissue. It is thought that most of the energetic needs of ruminal epithelial tissue is satisfied by the metabolism of butyrate (Van Houtert, 1993). The BHBA produced enters portal circulation and is used as a precursor for mammary synthesis of short-chain and medium-chain fatty acids that are secreted in milk. Very little acetate is metabolized by the rumen epithelia because of the rumen epithelia's lack of acetyl-CoA synthetase, which is vital for the metabolism of acetate (Van Houtert, 1993).

All VFA spared by the ruminal epithelia are then transported to the liver where most of the VFA are further metabolized (Van Houtert, 1993). Just like in the ruminal epithelia, acetate undergoes very little metabolism in the liver due to low activity of acetyl-CoA synthetase. Only 0.02% of the acetate absorbed from the rumen is used by the liver to synthesize carotenes, cholesterol, porphyrins and for de-novo synthesis of long-chain fatty acids (Bergman and Wolff, 1971; Van Houtert, 1993). Acetate is used by muscles tissue as an energy source, synthesis of long-chain fatty acids by adipose tissue, and de novo fatty acid synthesis in the mammary gland (Bell, 1979; Van Houtert, 1993). The BHBA that is derived from ruminal epithelia is also used for mammary gland fatty acid de-novo synthesis. Apart from the mammary gland, BHBA can be used by brain cells as an energy source. Propionate is mainly used for hepatic gluconeogenesis, providing up to 90% of ruminant's glucose requirements (Van Houtert, 1993).

2.3   Ruminal acidosis

The goal of dairy cow feeding is to provide enough nutrients to support milk production by increasing diet fermentation rates. This is accomplished by feeding diets that are progressively higher in grain content. Grains commonly fed like barley have high fermentable carbohydrate content, and their fermentation leads to the accumulation of VFA in the rumen. High VFA production is desirable in ruminants because it means there is more metabolizable energy available. However, VFA production leads to the release of protons which, in turn, may lower the pH in the rumen if the rate of VFA production is not matched by the rate of proton neutralization and VFA absorption (Yang et al., 1997; Iqbal et al., 2012). If the rate of VFA removal by the ruminal epithelia is low or if there is insufficient buffering, the drop in pH may cause ruminal acidosis (Beauchemin et al., 2006; Krause and Oetzel, 2006). Because of barley's high fermentation rates and high starch content, animals fed on barley-based diets are at a higher risk of ruminal acidosis compared to those fed other cereal grains.

Ruminal acidosis is a nutritional disorder which is characterized by rapid accumulation of acids in the rumen caused by an imbalance between fermentation acid production and acid neutralization and removal (Krause and Oetzel, 2006). Ruminal acidosis is triggered by either insufficient buffering caused by low dietary fibre, rapid intake of highly fermentable carbohydrates or inadequate ruminal adaptation to high fermentation rates (Krause and Oetzel, 2006). There are two main forms of ruminal acidosis, which are acute ruminal acidosis characterized by pH between

5.2 and 5.0, and sub-acute ruminal acidosis (SARA) which has pH ranges of between 5.8 and 5.5 (Aschenbach et al., 2011)

Acute acidosis is caused by the accumulation of lactic acid because of a shift in fermentation from VFA to lactic acid production, while SARA is caused by VFA accumulation (Nocek, 1997). Volatile fatty acid accumulation is caused by the rapid production of VFA which is not matched by absorption by epithelia cells or buffering (Nocek, 1997). The main form of ruminal acidosis found in dairy cows is SARA, while acute acidosis is more common in high concentrate fed beef cattle (Schwaiger et al., 2013).

2.3.1 Clinical signs of sub-acute ruminal acidosis

Usually cows affected by SARA do not show clear clinical signs, symptoms may be subtle and varied, and not all the animals in the herd may be experiencing SARA, with some of the symptoms only showing up weeks after the acidotic episode (Tajik et al., 2009). This makes diagnosis of ruminal acidosis difficult leading to high prevalence rates. Clinical signs of ruminal acidosis include anorexia, elevated temperature, diarrhea, lethargy, tachypnea, recumbence and even death, but final diagnosis is usually by measuring ruminal pH (Krause and Oetzel, 2006). Ruminal acidosis reduces productivity and is associated with laminitis (Nocek, 1997), reduced feed intake, reduced milk fat content (Kleen et al., 2003) and liver abscesses (Nagaraja and Lechtenberg, 2007). Ruminal acidosis also affects animal welfare because of its association with lameness, which is one of the main causes of early culling in dairy herds (Krause and Oetzel, 2006).

2.3.2 Prevalence and economic cost of sub-acute ruminal acidosis to dairy herds

Clinical signs for SARA are usually subtle, this poses difficulty in diagnosing the disease, and cows suffering from it can be overlooked. This causes SARA to have high prevalence rates in the farms causing financial losses. Research by Gao and Oba (2014) showed incidence rates of SARA between 19% and 26% during lactation. In another study in Wisconsin by Garrett et al., (1999) incidence rates of 20.1% in lactating cows were recorded. There has not been studies in Western Canada that I am aware of on SARA prevalence in dairy cows, but studies on feedlot steers have shown ruminal acidosis rates of up to 37.8% (Castillo-Lopez et al., 2014). Because of these high prevalence rates, ruminal acidosis causes significant financial losses to dairy farms. Ruminal acidosis causes losses of approximately US$475 per cow per year because of a decrease

in milk production of 3 kg per cow per day and decreases of milk fat and true protein content by 8.1% and 3.5%, respectively, and its estimated that the US dairy industry incurs loses between 500 million and 1 billion dollars due to SARA (Krause and Oetzel, 2006).

2.3.3 Factors affecting the severity of ruminal acidosis to dairy cows

The severity of ruminal acidosis is affected by the diet's forage to concentrate ratio. Diets with higher concentrate to forage ratio increase the frequency of ruminal acidosis episodes. In an experiment by Penner et al. (2007), prepartum cows fed a high concentrate diet experienced more episodes of ruminal acidosis and the episodes of ruminal acidosis lasted longer compared to cows fed a diet with less concentrates. In the same study, it was observed that cows undergoing transition had longer duration under the pH thresholds of 5.8, 5.5, and 5.2, showing that diet changes influence the severity of ruminal acidosis.

The type of grain fed to animals also affects the severity of ruminal acidosis because of differences in rates of fermentation between grains. A study by Chibisa et al. (2015) barley grain fed animals tended to have lower ruminal pH, and spent a longer period under the pH threshold of 5.8 compared to cows fed the less ruminally fermentable corn. Ruminal acidosis severity is also influenced by grain adaptation. Rapid shifts from less fermentable diet to highly fermentable energy dense diet can trigger ruminal acidosis. It is important to adapt the rumen to high energy diets so that the rumen is better capable to deal with increased lactate production and VFA concentrations. Adaptation involves gradually increasing the energy content which leads to changes in the rumen microflora (Tajima et al., 2000), and increased growth and proliferation of ruminal epithelia (Dirksen et al., 1985). After parturition dairy cows are at increased risk to ruminal acidosis because of abrupt changes from a high forage dry cow diet to a energy dense diet (Grant and Albright, 1995; Nocek, 1997), and ruminal acidosis is most prevalent after parturition (Gröhn and Bruss, 1990).

2.3.4 Bacterial changes associated with sub-acute ruminal acidosis

Ruminal acidosis leads to shifts in ruminal populations of microbes, mainly the proportions of amylolytic, simple sugar and lactate fermenting and utilizing bacteria (Nagaraja and Lechtenberg, 2007). The main bacteria associated with ruminal acidosis in animals not adapted to grain diets is *S. bovis* (Nagaraja and Lechtenberg, 2007). When ruminant animals are fed a diet high in fermentable carbohydrates, *S. bovis* populations rapidly increase, doubling every 12

minutes leading to rapid accumulation of VFA, lowering pH and creating an environment optimum for lactic acid producers like *lactobacilli* to proliferate while inhibiting the growth of most rumen microbes. The low ruminal pH also increases the population of lactic acid utilizers (Nagaraja and Lechtenberg, 2007). The most common genus of protozoa in grain adapted animals is *Entodinium, Entodinium* has a higher tolerance to low ruminal pH characteristic of grain fed ruminants compared to other protozoa genera (Towne et al., 1990; Franzolin and Dehority, 1996). Ciliated protozoa help regulate ruminal pH because they predate on bacteria reducing their population in the rumen (Bonhomme, 1990), leading to a reduction in fermentation rates (Mendoza et al., 1993). Low ruminal pH leads to an overall reduction in protozoa population, and acute ruminal acidosis usually leads to defaunation (Nagaraja and Lechtenberg, 2007).

2.3.5 Mitigation of SARA

Ruminal acidosis can be mitigated by increasing ruminal pH buffering. Salivary secretions contain buffers that help regulate ruminal pH, and can contribute up to 30% of the total ruminal buffering capacity (Allen, 1997). The contribution of salivary buffers to ruminal buffering capacity is related to a diet's forage content and the physical and chemical properties of the diet, which, in turn, determine the rate of chewing, rumination and salivation. Diets for high producing dairy cows are usually formulated to be high in fermentable carbohydrates and low in NDF, and the forage is usually short length to increase its digestibility, but this increases the cow's susceptibility to ruminal acidosis (Beauchemin et al., 2006). Long forages increase rumination and chewing time, leading to increased salivation and buffering. Longer forages also stimulate mixing of ruminal digesta which increases VFA removal from the rumen. Also, fibre has significantly slower degradation and fermentation rates compared to non-structural carbohydrates (Beauchemin et al., 2006). To reduce the risk of ruminal acidosis, the NRC (2001) guidelines state that at least 25% of the diet should be NDF, and 75% of the NDF should be of forage origin. Studies have shown a reduction in SARA when diets contain a minimum of 30-33% peNDF (Zebeli et al., 2008).

The main cause of ruminal acidosis is highly fermentable carbohydrate intake. It is recommended that to reduce ruminal acidosis, dietary content of NFC should be in the range of 35-40% DM in a high sugar or starch diet and 40 to 50% in diets high in other carbohydrates (Hoover and Miller., 1995). However, the strategies mentioned above can reduce VFA production in the rumen. Because VFA are the major source of energy for maintenance and productive

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functions in dairy cows, a reduction in ruminal VFA production would limit animal productivity. Another potential strategy to reduce ruminal acidosis without affecting productivity is the substitution of starch with simple sugars. Sugars are a class of carbohydrates that are water-soluble which includes the monosaccharides fructose, galactose and glucose, and the disaccharides lactose, maltose and sucrose (Oba, 2011).

2.4   Effect of partial substitution of starch with simple sugars on ruminal pH

Simple sugars are known to degrade faster than starch in the rumen (40-60 vs 20-40 Kd, %/h) (Van Amburgh et al., 2015), thus producing VFA at a faster rate than starch. Based on these differences in ruminal fermentation rates, it would be anticipated that ruminal pH of animals fed sugars would be lower compared to animals fed grain starch; however, numerous studies have demonstrated that is not the case (Oba, 2011). Numerous studies have shown that when starch is substituted with sugar there is no significant change in ruminal pH despite the increased fermentation rates (Defrain et al., 2004; Vallimont et al., 2004). In fact, a study by Penner et al. (2009) showed an increase in ruminal pH when cracked corn was partially substituted with sucrose.

Gäbel et al. (1991) suggested that changes in proportions of VFA production when diets increase fermentability may increase the permeability of ruminal epithelia to VFA. Sugar inclusion has been shown to increase the rate of butyrate production in the rumen (DeFrain et al., 2006; Chibisa et al., 2015; De Seram, 2016). When butyrate is absorbed into the ruminal wall, most of it is metabolized into BHBA to provide energy for ruminal epithelia (Weigand et al., 1975). Increasing butyrate metabolism is known to stimulate growth and proliferation of ruminal epithelia (Tan and Murphy, 2004) through the effects of IGF-1 and EGF (Penner et al., 2014). Increases in ruminal epithelia cell surface area can help explain why pH does not decrease on high sugar diets. There is also evidence that shows an increase in the expression of ruminal epithelial transport proteins. Chibisa et al. (2015) reported that when lactose partially replaced corn and barley grain there was an increase in Cl^--competitive absorption rates of propionate and acetate. Cl^--competitive absorption also increases HCO_3^- secretion into the rumen, which could potentially increase ruminal buffering capacity (Chibisa et al., 2015). This means that partial replacement of grain starch with simple sugars can be used to increase diet ruminal fermentability and energy supply without increasing the risk of ruminal acidosis.

Feedstuffs that are high in simple sugars include certain high sugar cultivars of ryegrass and alfalfa, and industrial byproducts like citrus pulp, molasses, whey permeate and corn steep liquor (Oba, 2011). Whey permeate is of importance because it is readily available locally from the Saputo milk processing plant in Saskatoon, where it is produced as a byproduct of cheese production. Whey permeate contains approximately 70% lactose on a dry matter basis (Oba, 2011), so it is a rich source of sugar.

There has been studies that have looked at dietary inclusion of liquid whey, whey permeates and pure lactose on ruminal pH in dairy in the past. Chibisa et al. (2015) partially replaced corn starch and barley grain with dried whey permeate (DWP) up to 6%, without any reduction in ruminal pH. Similarly Defrain et al. (2004) fed dairy cows diets with either liquid whey, low or high pure lactose (5.3, 6.1 and 13% lactose content respectively) without a drop in pH. Seram, (2016) also replaced corn starch with DWP levels of up to 11.5% and ruminal pH was not lowered. It is noteworthy that in both studies by Defrain et al. (2004) and De Seram (2016), ruminal pH was recorded using spot pH measurement, which limits data interpretation because the pH recordings do not properly report all the changes induced by the diet. These studies seem to suggest that partial replacement of barley grain with DWP to levels supplying 13.5% lactose can help reduce the risk of ruminal acidosis, but there has not been a comprehensive study where ruminal pH was continuously recorded in diets where DWP was included in barley grain diets typically fed in Western Canada up to 11.5%. A major objective of my thesis research was to determine the effects of partial substitution of dietary starch with sugars on ruminal pH using continuous pH measurements to determine the extent of ruminal acidosis.

2.5 Effect of partial substitution of starch with simple sugars on animal productivity

Many published studies have investigated the feasibility of feed ingredients high in simple sugars to ruminants (Kellogg and Owen, 1969; Vallimont et al., 2004; DeFrain et al., 2006; Penner and Oba, 2009). Partial replacement of grain starch with simple sugars can be used as a strategy to improve DMI, nutrient digestibility, and productivity. Penner and Oba (2009) partially replaced corn grain with 4.7% DM sucrose to achieve a dietary ethanol soluble carbohydrate (ESC) content of 8.7% (versus 4.5% in the control diet), which increased DMI by 1 kg/d, and also tended to increase milk fat yield in dairy cows. Similarly, Broderick et al. (2008) observed a linear increase in DMI with increased sucrose inclusion in the diet (0, 2.5, 5.0, and 7.5% sucrose DM), and Broderick and Radloff, (2004) observed linear and cubic increase in DMI with dietary inclusion of dried and liquid molasses (0, 4, 8 and 12% of either dried or liquid molasses DM).

Lactose has also been shown to have the potential for use as a source of energy for cattle. DeFrain et al. (2004) fed dairy cows diets with either liquid whey, low or high pure lactose (5.3, 6.1 and 13% lactose content respectively), as a replacement of corn starch, which increased DMI of the cows. Steers have been fed diets with up to 60% liquid whey on a dry matter basis without negatively affecting digestion or growth (King and Schingoethe, 1983; Susmel et al., 1995). Chibisa et al. (2015) partially replaced either corn or barley grain with DWP up to 6% and observed improved apparent total tract DM digestibility without negatively affecting ruminal pH. Seram, (2016) also partially replaced barley grain with DWP up to 11.5% and there was no difference between the diets with or without added DWP on overall productivity in lactating Holstein cows.

Evidence from these experiments seems to suggest that dietary inclusion of DWP can be used to increase ruminal fermentation rates leading to a potential increase in DMI, nutrient digestibility and milk production without increasing the risk of ruminal acidosis.

2.6 Effect of partial substitution of starch with simple sugars on volatile fatty acid production

Effects of simple sugars on ruminal VFA profile have been variable in studies in the past. Increasing dietary lactose content up to 14.3% increased the ruminal concentration of butyrate while decreasing concentrations of acetate and branched chain VFA in an experiment by DeFrain et al. (2004). Similarly, Broderick et al. (2008) reported a decrease in the ruminal concentration of branched-chain VFA, but no change in ruminal butyrate concentration in cattle fed a diet containing up to 7.5% DM sucrose. Vallimont et al. (2004) also replaced grain starch with sucrose up to 7.5% in continuous culture fermenters and noticed an increase in butyrate and isobutyrate concentration, and a tendency for a decrease in valerate concentration. Chibisa et al. (2015) also saw increases in ruminal butyrate concentration with DWP inclusion. Moreover, Seram, (2016) increased dietary DWP content and ruminal butyrate concentration increased cubically. Studies that included lactose have consistently noticed increases in butyrate concentration (DeFrain et al., 2004; Chibisa et al., 2015; De Seram, 2016) unlike studies with other simple sugars. Increased butyrate concentration is known to stimulate growth and proliferation of ruminal epithelia (Tan and Murphy, 2004); this can potentially help maintain ruminal pH at optimum levels and reduce the animal's susceptibility to ruminal acidosis.

Differences observed between studies on the effects of simple sugars on VFAs is probably due to different dietary compositions, sample and data collection protocols, and inclusion levels of simple sugars between studies. Also, the concentration in the rumen does not translate to production and the differences in concentrations may be due to differences in the rate of VFA production and removal from the rumen (Oba, 2011).

2.7   The efficiency of nitrogen utilization in dairy cows

The N contained in dairy diets as true protein or NPN (through microbial protein synthesis) is important because it supplies amino acids (AA) that are the precursors for milk protein synthesis (Arriola Apelo et al., 2014). However, the efficiency of utilization of dietary N is very low with only 20 to 35% of dietary N being incorporated into milk N (Arriola Apelo et al., 2014), and up to 80% of dietary N can be lost in urine and feces (Mulligan et al., 2004). Efficiency of conversion of feed N into milk crude protein is poor for many reasons, including low diet readily fermentable carbohydrate content, high diet ruminally degradable protein content, or the lack of synchrony between energy availability (from carbohydrate fermentation) and N availability (from crude protein fermentation) for efficient microbial protein synthesis (MPS) (Tan and Murphy, 2004). This is particularly so because dairy cows are fed high forage diets that contain fermented forages (silages) which are high in RDP. When the RDP is fermented in the rumen without a matching supply of ruminally-fermentable carbohydrate, the asynchronous availability of N and energy results in increased rumen ammonia (NH_3) concentrations, increased NH_3 absorption into the blood, and conversion into urea by the liver and excretion in urine (Mccormick et al., 2001). Urinary N excretion represents an irreversible loss of N for the dairy cow. Increasing carbohydrate fermentation has been shown to increase synchrony, thereby leading to more efficient utilization of RDP by ruminal microorganisms (Broderick and Radloff, 2004).

2.7.1   Sustainable dairy agriculture

Nitrogen is mainly lost from the animal in the form of urinary urea-N and fecal N. Nitrogen excretion is a concern because of its environmental impact, it has been linked to eutrophication, global warming, coastal hypoxia, and acidification of rain, soil and surface water (Arriola Apelo et al., 2014). Animal farming has been estimated to contribute an average of 9% of all greenhouse emissions (O'Mara, 2011). Among all the animal farming activities dairy farming produces the most nitrogenous waste that leads to environmental pollution (MAFF Environment,

1994). Apart from environmental pollution, N loss also has impacts on human health because it aggravates asthma and pollutes drinking water (Arriola Apelo et al., 2014). Also, N supplements in diets are the most expensive, N losses represent economic losses to dairy farmers. Excreted N volatilizes into NH_3, urinary N has a higher rate of NH_3 production because of its high urea-N content as compared to fecal N (Varel et al., 1999). Urinary urea is the main source of NH_3 volatilization (Varel et al., 1999) and there is a need to reduce urinary urea output. The environmental impacts of animal production are expected to increase because of projected increases in demand for animal products. Demand and production of milk and beef is expected to double in the next half-century, driven by increasing global population and increased average income, particularly in the developing world (Poulsen et al., 2013). Sustainability of livestock production can be improved by increasing N utilization by either reducing ruminal degradation of dietary N, or by increasing urea-N recycling to the rumen.

2.7.2 Ruminal degradation of dietary proteins

Ruminal degradation of proteins is accomplished by a consortium of microbes working symbiotically by producing complimentary enzymes that help in the degradation of proteins (Bach et al., 2005). These synergies are necessary because not all microbes produce the complete sets of proteases needed to break down all the bonds present in protein (Wallace et al., 1997). Bacteria, protozoa, and fungi all participate in microbial protein degradation, but most of ruminal protein degradation is due to the activities of proteolytic bacteria (NRC, 2001), which represent 40% of ruminal bacterial species that have been isolated (Wallace, 1996). Protozoa also play a significant role in protein degradation, and defaunation has been shown to lead to a reduction in total tract protein digestibility in a meta-analysis by Williams and Coleman (1992), but their role is minor because they are less numerous than bacteria (Jouany, 1996).

Ruminal bacteria produce a diverse variety of proteases and peptidases, which help hydrolyzes the bonds between AA in protein to produce free AA and peptides (NRC, 2001). The first step in bacterial degradation of protein is the attachment of bacteria to feed particles, which brings cell-bound proteases closer to feed particles (Figure 2.3) (Bach et al., 2005). Ruminal microbes also produce extracellular proteases, but 90% of proteases are cell-bound
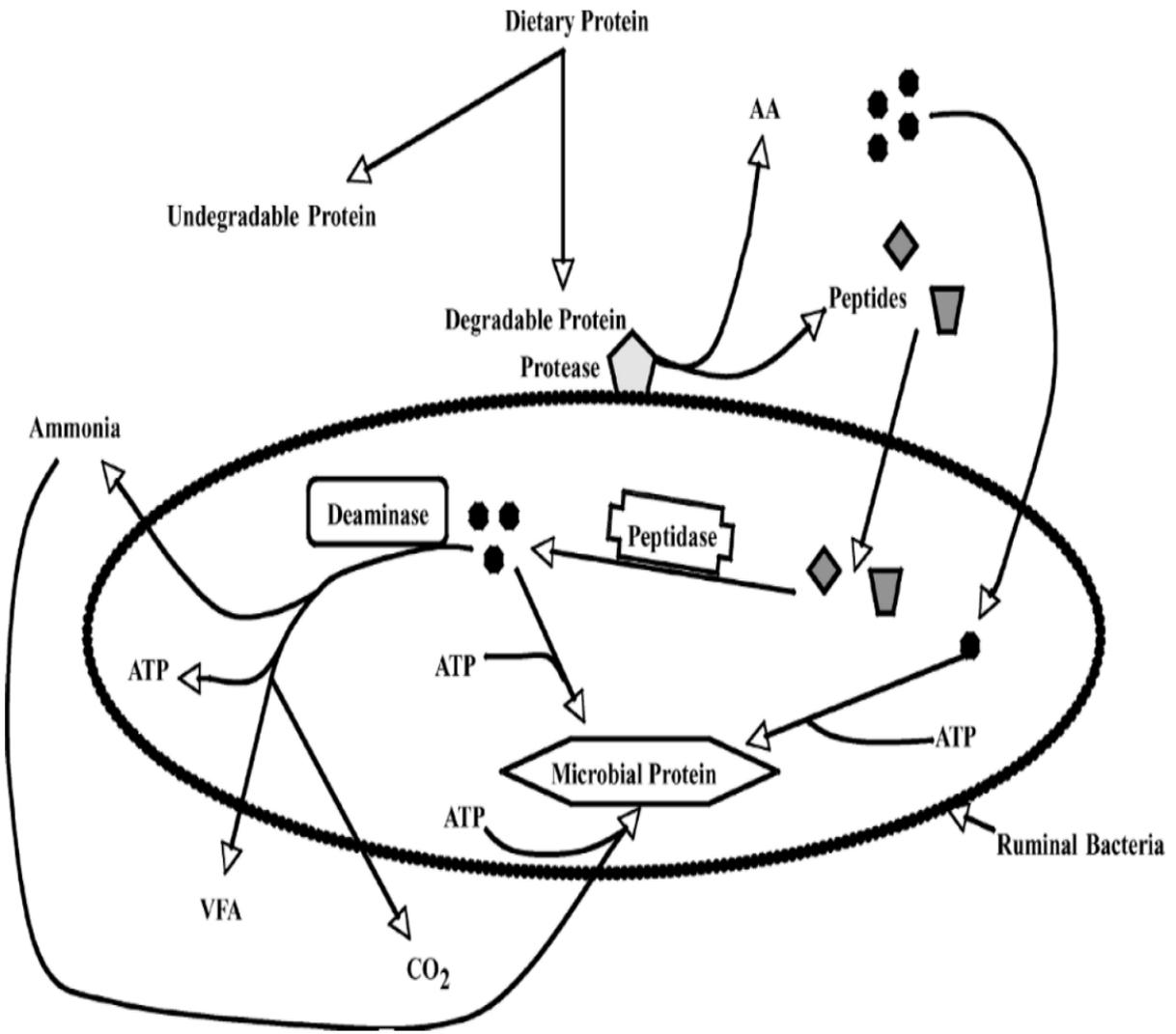
Figure 2.3. Ruminal degradation of protein (Bach et al., 2005)

(Broderick et al., 1991). Proteases and peptidases produced by ruminal microbes hydrolyze the bonds between AA in protein to produce free AA and peptides (Wallace, 1996; Bach et al., 2005), and these are actively absorbed into microbial cells through transport proteins, peptides are further digested into AA inside microbial cells (Tamminga, 1979). Free AA inside microbial cells are used for protein synthesis. Ruminal microbes usually degrade and absorb more AA than they can utilize, because bacteria cannot transport AA out of their cells, excess AA is deaminated into methane, NH_3, VFA, and CO_2 (Tamminga, 1979). The free NH_3 produced is also used to resynthesize microbial protein, and excess NH_3 is excreted out of the cell together with the other products of deamination. If the rate of protein degradation is faster than microbial protein synthesis, NH_3 accumulates in the rumen (NRC, 2001), which reduces N utilization efficiency.

2.7.3 Ruminal microbial protein production

Ruminal microbial protein is derived from bacteria, fungi, and protozoa that grow in the rumen. Microbial protein plays a significant role in ruminant animal's N supply (Figure 2.3). Microbial protein is highly digestible compared to dietary proteins, with an average digestibility of 85% (Storm and Ørskov, 1983). Additionally, microbial protein is of high quality because its AA composition which matches lean muscle and milk composition (Orskov, 1992) with a higher content of methionine and lysine compared to high-quality dietary protein (Jasim et al., 2015). Because of its superior quality, microbial protein can contribute between 60 to 80% of AA available post-ruminally (Jasim et al., 2015). To increase animal productivity, it is important to maximize microbial protein synthesis.

The bulk of microbial protein available post-ruminally is of bacterial origin. The contribution of protozoa to microbial protein production is low, protozoa contribute up to 40% of rumen microbial biomass (Russell and Rychlik, 2001), but they only contribute 11% of microbial protein available post-ruminally (Shabi et al., 2000). Protozoa's low contribution to N flow out of the rumen is because they have low passage rates (Bach et al., 2005).

2.7.3.1 Factors affecting microbial protein production

Microbial protein production has a significant impact on animal productivity, accounting for up to 65% of variability in milk production (Schwab and Ordway, 2004). Several factors influence microbial protein synthesis, but for this study I am only reviewing the effects of dietary

supply of RDP, fermentable energy, and the synchrony between protein and energy fermentation on microbial protein synthesis.

2.7.3.1.1 Ruminally fermentable carbohydrates

Microbial protein synthesis is dependent mainly on the availability of energy in the rumen (Bach et al., 2005). Ruminal fermentable energy which is usually in the form of carbohydrates powers MPS and bacterial growth (Koening et al., 2003). Ruminal microbes also use carbohydrates as carbon skeletons for AA synthesis together with NH_3 (Bach et al., 2005). Increasing energy supply to ruminal microbes by increasing overall dietary carbohydrate content or increasing diet fermentability has been shown to increase MPS and utilization of recycled urea-N by microbes. Zhou et al. (2015) increased energy supply to ruminal microbes by either increasing ruminal corn starch digestibility by processing (ground vs steam flacking) and overall dietary corn starch content, both increasing dietary starch content and steam flacking increased MPS and overall animal productivity in lactating dairy cows. Gozho et al. (2008) also investigated the effects of feeding grains with different fermentation rates on animal productivity; barley grain fed cows had 48 g/d higher microbial N flow out of the rumen compared to cows fed the less ruminally fermentable oats.

Simples sugars also have the potential to support more microbial growth because of their higher degradation rates compared to grain starch or structural carbohydrates (Van Amburgh et al., 2015). Bailey et al. (2012) ruminally dosed steers with glucose once a day which reduced ruminal NH_3 concentration, ruminal NH_3 concentration is a good indicator of MPS. Supplementing glucose to the steers diet also increased microbial use of recycled urea-N. Similarly, Chibisa et al. (2015) and Seram, (2016), fed DWP to lactating dairy cows, which reduced ruminal NH_3 concentration.

Although increasing dietary fermentable carbohydrates supply increases MPS, if the supply of fermentable carbohydrates exceeds the needs of ruminal microbes, MPS efficiency is reduced because of increased energy spilling (Hackmann and Firkins, 2015).

2.7.3.1.2 Rumen degradable protein supply

Rumen degradable protein is the portion of dietary CP that is degraded by ruminal microbes, and supplies microbes with the AA, NH_3, and peptides necessary for MPS and growth. Past studies have shown that increasing dietary RDP content increases MPS. Wickersham et al.

(2008) supplemented RDP to steers in the form of casein, which increased ruminal NH_3 concentrations and this led to increased duodenal microbial-N flows and increased use of recycled urea-N by ruminal microbes. Bailey et al. (2012) also supplemented steers with casein, which also led to an increase in ruminal NH_3 and MPS.

The increased ruminal NH_3 concentration witnessed with increasing dietary RDP content is important because ruminal NH_3 can supply N for microbial growth for up to 80% of ruminal microbes (Nolan and Leng, 1972). It is essential to supply enough NH_3 to microbes to maximize MPS. Reynal and Broderick, (2005) fed lactating cows feed with either 10.6, 11.7, 12.3 and 13.2% RDP and concluded that ruminal microbes need at least 11.8 mg/dL NH_3 not to suppress MPS. For optimum MPS, the NRC, (2001) recommends that dietary RDP content be maintained between 9.5% and 11.5%. In an experiment by Reynal and Broderick, (2005), MPS plateaued at 12.3% dietary RDP content, and MPS efficiency decreased after that. It is vital to only supply enough RDP to supply N needs for microbes because oversupply of RDP increases microbial energy spilling and reduces the efficiency of N utilization. According to the NRC (2001), milk production is maximal at 12.2% dietary RDP content.

2.7.3.1.3 Synchrony between protein and energy fermentation

Nutrient synchrony is a state where supply of N and energy is in the proportions needed by ruminal microbes, and it is achieved by matching the rates of degradation of carbohydrates and N. Theoretically nutrient synchrony is hypothesized to improve feed conversion efficiency, MPS, post-ruminal nutrient supply, reduce ruminal NH_3 and excretion of urinary urea-N, leading to improved animal performance (Hall and Huntington, 2008; Hersom, 2008). The efficiency of conversion of feed N into milk N is poor because of the lack of synchrony between carbohydrate and N fermentation. Dairy cows are fed diets that contain silages which are high in RDP. When RDP is degraded in the rumen without a matching supply of ruminally-fermentable carbohydrate, the asynchronous availability of N and energy results in increased rumen NH_3 concentrations, increased NH_3 absorption into the blood, and conversion into urea-N by the liver and excretion in urine (Mccormick et al., 2001).

Synchronization of N and carbohydrate degradation rates has been shown to improve N utilization. Davies et al. (2013) experimented with feeding either a high or low RDP, and either high or low ruminally digestible starch and observed microbial protein increased with increasing

RDP content on the high ruminally digestible starch diet but RDP levels had no effect on the low ruminally digestible starch diet.

Nutrient synchrony's practical applicability is limited because of the vast amount of factors that influence nutrient synchrony, including difficulty in correctly predicting nutrient availability of different feed ingredients, effect of ruminal parameters like pH on nutrient availability, differences between individual animals in their ruminal microflora and their nutrient requirements and impact of urea-N recycling (Cole and Todd, 2008; Hall and Huntington, 2008). Results from experiments that sought to synchronize rates of protein and carbohydrate availability have been mixed with some recording positive results (Kim et al., 1999; Bailey et al., 2012; Davies et al., 2013), and others showing no effect (Rotger et al., 2006; Valkeners et al., 2006). This current study will investigate the effect of synchronizing energy fermentation rates (by adding lactose in the form of dried whey permeate) and protein degradation rates (by altering dietary RDP content using heat-treated soybean meal) on productivity and N efficiency.

2.8 Urea-N recycling in dairy cows

Ruminants synthesize substantial amounts of urea in the liver from portal NH_3 and hepatic AA deamination, and the quantity of urea-N produced can be greater than dietary digestible N intake, and if all the urea-N is excreted in urine the animal would be in negative N balance (Lapierre and Lobley, 2001). However not all the urea synthesized by hepatic tissue is excreted in urine, between 40 to 80% of the urea synthesized by the liver is recycled back to the GIT (Huntington, 1989). Recycled urea-N contributes a lot to available N in the rumen and can increase the digestible N inflows in the rumen of lactating cows by as much as 60% (Lapierre and Lobley, 2001). Urea-N recycling helps ruminants improve N utilization by reducing excretion of N in the form of urinary urea-N, increasing microbial N synthesis in times of deficit and maintaining positive N balance. Increasing endogenous urea-N recycling can help reduce overall N intake and excretion, which can help reduce the overall environmental impact of animal farming and cost of production.

2.8.1 Rumen ammonia production

Ammonia is the main product of ruminal N degradation, and NH_3 is the main source of N utilized by ruminal microbes for protein synthesis (Nolan and Leng, 1972). Ruminal NH_3 is produced by microbes from degradation of dietary N and endogenous urea-N (Abdoun et al.,

2006). Ammonia production in the rumen often exceeds the capacity for ruminal microbes to utilizes for MPS, leading to NH_3 accumulation and removal from the rumen (Tan and Murphy, 2004). Ruminants absorb a large amount of their N in the form of NH_3, and in some diets more N is absorbed from the GIT in the form of NH_3 than as AA (Reynolds, 1992). Ammonia that is not used for MPS is removed from the rumen by passage out of the rumen or absorption into portal circulation, with absorption removing the highest amount (35-65% vs 10%) (Abdoun et al., 2006).

Ruminal ammonia occurs in two forms, the non-ionized from NH_3, and the ionized from NH_4^+. The NH_3 form is lipid soluble and is absorbed from the rumen by passive diffusion. ~~The~~ rate of diffusion is influenced by ruminal NH_3 concentration, and ruminal pH, and is predominant at pH above seven (Walter and Gutknecht, 1986). The main form of ammonia present in the rumen at low pH is NH_4^+, and most ammonia removed from the rumen is in this form (Abdoun et al., 2006). The NH_4^+ form of ammonia is not lipid soluble and its absorption is via potassium channels on the ruminal epithelia cells (Stumpff et al., 2009).

The rate of NH_3 absorption is positively correlated to NH_3 concentration in the rumen and dietary factors that affect ruminal concentration of NH_3 also affect NH_3 absorption from the rumen. The main dietary factors that influence ammonia absorption are the availability of fermentable energy and rate of protein degradation in the rumen (Reynolds and Kristensen, 2008).

2.8.2 Production of urea

Ammonia concentration above 0.5 mg dL^{-1} in extrahepatic tissue is toxic, and its symptoms include permanent brain damage leading to a comma and sometimes death (Singer, 2003). To solve this problem, the body converts blood NH_3 into a less toxic organic compound, urea (Stewart and Smith, 2005). Ammonia is converted into urea in the mitochondrion matrix and cytosol of hepatocyte cells of the liver, via a series of enzymatic reactions (Figure 2.4).

In short, NH_3 reacts with CO_2 or HCO_3 to produce carbamoyl phosphate in the periportal hepatocyte cell mitochondrial matrix; the carbamoyl group is then transferred to ornithine to form citrulline. Citrulline is translocated into the hepatocyte cell cytosol where it combines with aspartame to form arginosuccinate which is then cleaved into fumarate and arginine. Arginine is then finally hydrolyzed into urea with the regeneration of ornithine. The urea synthesized by the liver is released into the blood and is either secreted into the GIT or excreted in urine.

$NH_3 + CO_2 + 2ATP$

Carbamoyl
phosphate

Urea

Ornithine

MG^{2+}

H_2O

Arginase

Ornithine
carbamoyl
transferase

Arginase

Citrulline

AMP

Urea

Arginosuccinate
lyase

Arginosuccinate
synthetase

Arginosuccinate

ATP

Aspartate
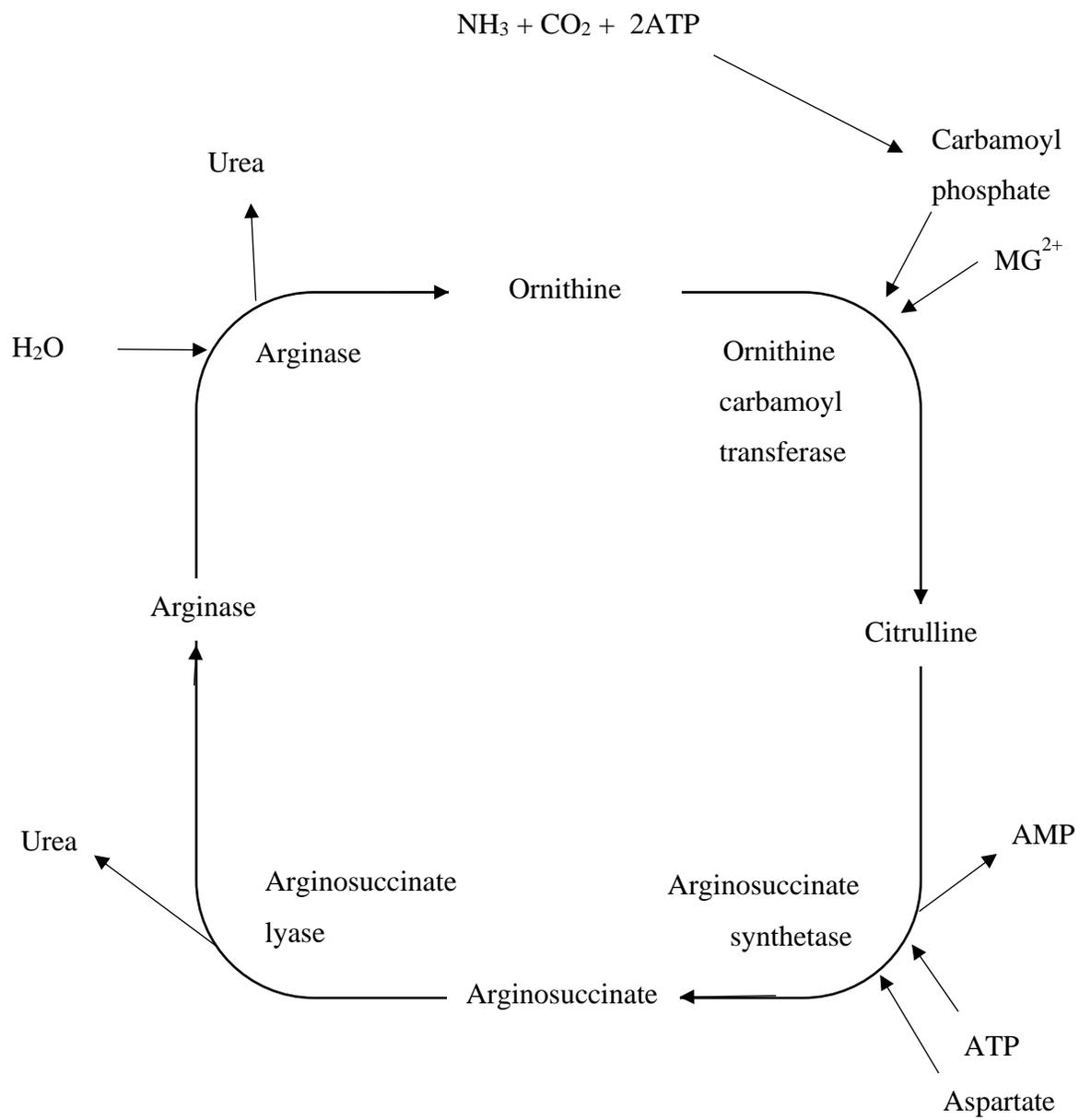
Figure 2.4 The ornithine cycle

2.8.3 Urea-N recycling to GIT

Urea-N synthesized by hepatic tissue can potentially be recycled back to the rumen via saliva or by diffusion through the GIT epithelia. Endogenous urea-N present in saliva is secreted by the salivary glands, endogenous urea-N transfer to the GIT via saliva is mainly affected by the physical characteristics of the diet. Forage-based diets have a higher rate of endogenous urea-N recycling via saliva compared high concentrate diets because of increased chewing and salivation. In a study by Huntington, (1989) feedlot steers fed a high concentrate diet had 23% of total endogenous urea-N recycled to the rumen through saliva compared to 69% in steers fed a high forage diet. Endogenous urea-N is recycled back to the GIT by simple lipid diffusion across the GIT epithelia this is facilitated by urea transporter proteins (UT-B) and aquaporins (AQP-3, 7, and 10) present in GIT epithelia (Abdoun et al., 2006; Walpole et al., 2015). Endogenous urea-N is recycled to the whole GIT, but the rumen is where urea-N is more efficiently utilized to form microbial protein (Lapierre and Lobley, 2001); therefore it is important to maximize urea-N recycling to the rumen. Factors affecting urea-N recycling to the rumen are discussed in the next section.

Endogenous urea-N recycled to the rumen is hydrolyzed into NH_3 and CO_2 by urease produced by ruminal bacteria. The NH_3 can then be either utilized by ruminal microbes for MPS, reabsorbed by the ruminal epithelia or passed out of the rumen. It is important to improve urea-N recycling to improve N utilization in ruminants.

2.8.3.1 Factors affecting urea-N recycling to the rumen

Urea-N recycling to the rumen and its fate in the rumen is affected by many interrelated factors which include dietary CP content, DMI, forage to concentrate ratio, ruminal VFA concentration, urease activity and ruminal pH.

Ruminally degradable protein

Dietary RDP supply to the rumen determines ruminal NH_3 concentrations. Increasing RDP supply to the rumen increases ruminal NH_3 concentration (Bailey et al., 2012; Mutsvangwa et al., 2016), leading to increased transfer of ruminal NH_3 into portal circulation (Walter and Gutknecht, 1986), increased endogenous urea-N synthesis by the liver, increased blood urea-N concentration, increased urinary urea-N excretion, and increased urea-N recycling to the GIT in absolute amounts. Wang et al. (2012) fed weathers either a high, medium or low RDP diets, increasing

dietary RDP content increased ruminal NH_3, plasma urea-N concentrations, urea-N recycling, and proportion of ruminal NH_3 derived from plasma urea-N. Even though low dietary RDP content reduces urea-N transfer to the GIT (g/d), low dietary RDP increases fractional transfer rates of endogenous urea-N into the GIT. Low dietary RDP content leads to low ruminal NH_3 concentrations, which help maintain a favourable urea concentration gradient between the blood and rumen lumen. This concentration gradient is maintained by microbial urease, an enzyme produced by epimural bacteria that converts urea into NH_3 and CO_2, ureases activity is reduced by increasing NH_3 concentration (Cheng and Wallace, 1979; Rémond et al., 1996). Ammonia also modulates urea transporter proteins, by altering luminal pH of epithelial cells. increased NH_3 increases luminal pH, which in turn reduces the permeability of the epithelia wall to urea (Lu et al. 2014).

Bailey et al. (2012) dosed steers on a low CP diet (5.8%) either 120g or 240g casein (a source of RDP) daily through the rumen cannula, steers dosed 120g of casein had lower ruminal NH_3 concentrations compared to those dosed 240g, and had higher fractional transfer rates of UER to the GIT. Also, the steers dosed with 240g casein also had higher UUE and UFE in absolute amounts; this suggests that lower dietary RDP content in low CP diets increases N utilization and efficiency of urea-N recycling. Wickersham et al. (2008) also dosed steers on a low CP diet (4.9%) increasing amounts of casein, increased casein infusion also increased ruminal NH_3 concentrations, leading to increased hepatic urea synthesis, excretion of urinary urea-N, and transfer of urea-N to the GIT in absolute amounts. However, increasing casein dosing reduced the proportion of endogenous urea transfer to the GIT (98.9% vs. 95.9% between the highest casein dose and lowest casein dose), also increasing casein infusion increased UUE and UFE. Rémond et al. (2009) altered supply of RDP to sheep by feeding either extruded peas (low in RDP) or raw peas, extrusion decreased ruminal NH_3 concentration, but there was no change in urea-N production or transfer rates of urea-N (g/d) to the GIT. Sheep fed the extruded diet had a higher proportion of endogenous urea-N transfer to GIT as a proportion of total urea-N production compared to sheep on a raw pea diet (72% vs. 52%).

Supply of fermentable energy

Increasing supply of fermentable carbohydrates is known to increase endogenous urea-N recycling to the GIT (Kennedy and Milligan, 1980; Huntington, 1989; Rémond et al., 1996).

Microbial protein synthesis and growth is energy dependent, and increasing dietary fermentable energy supply is known to increase NH_3 use by ruminal microbes in the synthesis of microbial protein (Nocek and Tamminga, 1991). Decreased ruminal NH_3 increases the rate of endogenous urea-N recycling to the GIT as discussed in previous sections, through its effects on bacterial urease and ruminal epithelia permeability. Increasing dietary fermentable energy supply also increases the rate of endogenous urea-N recycling through the action of SCFA and CO_2. Increased ruminal carbohydrate fermentation increases SCFA and CO_2 production, SCFA and CO_2 are absorbed into epithelia cells where they alter luminal pH which increases the permeability of the ruminal wall to urea, leading to increased transfer of endogenous urea-N into the rumen (Abdoun et al., 2010; Lu et al., 2014). Supply of fermentable energy can be increased by increasing the overall dietary readily fermentable energy supply or feeding carbohydrates with a faster rate of fermentation.

Theurer et al. (1999) increased urea-N recycling to the gut by increasing starch fermentation rates by steam flacking corn (compared to dry rolled corn). Similarly, Gozho et al. (2008) increased barley's ruminal fermentability by dry rolling barley compared to pelleted barley, which again increased urea-N recycling. These experiments show that urea-N recycling back to the rumen can be improved by increasing diet fermentability.

2.9 Effect of partial substitution of starch with simple sugars on the efficiency of nitrogen utilization and urea-N recycling

Simple sugars have a higher fermentation rate compared to other carbohydrates factions (Van Amburgh et al., 2015) therefore it is expected that partial substitution of grain starch with simple sugars would increase microbial protein synthesis, urea recycling, and overall N utilization. Simple sugars are expected to increase MPS because of increased energy supply compared to grain starch. The Cornell Net Carbohydrate and Protein System shows that ruminal microbes that utilize simple sugars produce 18% more microbial protein than microbes that utilize starch (NRC, 1996). This is further supported by Chamberlain et al. (1993), who increased MPS in sheep by supplementing with sucrose. Similarly, ruminal infusion of glucose (Rooke et al. 1987), or sucrose (Kim et al., 1999) in cattle has been shown to increased MPS. Moreover, in another study by Bailey et al. (2012), glucose supplements increased microbial use of recycled urea-N.

Increasing diet fermentation is known to increase urea recycling (Kennedy and Milligan, 1980), so it is expected that simple sugars should increase urea-N recycling since they have a higher degradation rate compared to other carbohydrate fractions. Additionally, simple sugars also increase ruminal butyrate concentration (DeFrain et al., 2006; Chibisa et al., 2015); butyrate is known to increase epithelial cell proliferation and growth (Penner et al., 2014), which may enhance urea recycling to the rumen by increasing surface area for urea-N transfer. However, there is very little direct evidence of improved N utilization and urea recycling with increasing sugar content in the diet. Broderick et al. (2008) observed a reduction in ruminal NH_3 and excretion of urinary urea-N and total N. Bailey et al. (2012) also increased overall fermentable energy supply by supplementing steers with 600g of glucose daily, this led to decreased ruminal NH_3 concentrations and reduced plasma urea-N, which tended to increase the proportion of endogenous urea-N recycled back to the rumen. In a study to investigate the effects of sugar supplementation in the form of molasses on the productivity of dairy cows, substitution of grain starch with molasses reduced concentrations of NH_3 in the rumen and excretion of N in the urine (Broderick and Radloff, 2004). Chibisa et al. (2015) partially replaced grain starch with DWP, which also reduced ruminal NH_3 concentrations, plasma urea-N, and milk urea-N. De Seram (2016) also partially replaced barley grain with DWP and ruminal NH_3 concentration also decreased.

These studies seem to suggest that the inclusion of simple sugars in ruminant diets may improve N utilization by increasing MPS, urea-N recycling, and reducing excretion of urinary urea-N. One of the current study's focus is to delineate the effects of partial replacement of barley grain with DWP on nitrogen utilization and urea recycling in lactating dairy cows.

2.10 Hypothesis

The hypotheses for the proposed research were two-fold:

1. That changing the proportion of dietary CP that is degraded in the rumen (by manipulating dietary RDP level) will alter urea-N recycling to the rumen and this effect will be more pronounced when more ruminally-fermentable energy is provided via the partial replacement of barley grain with dried whey permeate; and

2. That the replacement of barley grain with dried whey permeate will attenuate ruminal acidosis via changes in ruminal SCFA absorption.

2.11 Objectives

To delineate the interactions between partial replacement of barley grain with dried whey permeate (a source of lactose) and dietary rumen-degradable protein levels on feed intake, milk production, ruminal fermentation characteristics (ruminal pH, SCFA and ammonia-N concentrations), whole-body urea-N kinetics, nitrogen balance, and total-tract nutrient digestibility.

3 MATERIALS AND METHODS

3.1 Animals and experimental design

Eight lactating Holstein cows (738 ± 98 kg BW; 93 ± 39 DIM at the beginning of the experiment) were used in a replicated 4 x 4 Latin square design experiment with a 2 x 2 factorial arrangement of dietary treatments and 28-d periods (18 d of dietary adaptations and 10 d of data and sample collection). One square had four ruminally-cannulated cows for measurement of ruminal fermentation characteristics (ruminal pH, and SCFA and ammonia-N concentrations), whole-body urea-N kinetics, nitrogen balance, and total-tract nutrient digestibility. The experiment was conducted in the metabolism wing of the Rayner Dairy Research and Teaching Facility (RDRTF) at the University of Saskatchewan. Experimental cows were individually housed in tie-stalls and were handled and cared for in accordance with the Canadian Council on Animal Care (2009) regulations and their use in the experiment was approved by the University of Saskatchewan Animal Care Committee UCACS (Protocol No. 20040048).

3.2 Experimental treatments

The dietary treatment factors were: 1) two levels of dietary inclusion of whey permeate (0 vs. 11.2%, with the 0% diet being the standard barley-silage based dairy diet that is fed in the RDRTF): and 2) two levels of dietary RDP (9.5% vs. 11.5%) (Table. 3.2). The 9.5% RDP inclusion level is considered borderline deficient in terms of NRC (2001) recommendations, and the 11.5% RDP inclusion level is considered adequate. Dietary RDP level was manipulated by feeding heat-treated soybean meal (SoyPlus; Landus Cooperative, Ames, Iowa) or untreated soybean meal. Rumen degradable protein contents of SoyPlus and soybean meal were measured using the in-situ technique. Briefly, 5g samples of soybean meal and SoyPlus meal were weighed into nylon bags with a pore size of 40 µm. The samples were incubated in the rumen of lactating dairy cows fed the standard TMR fed at the RDRTF for 0, 2, 4, 8, 12, 24, 36, and 48 h using the gradual-addition/all-out schedule. After sample incubation, the nylon bags were rinsed with cold water until the water was clear. The zero-hr bags were also washed at the same time as the incubated bags. After washing, the bags were dried at 50°C for 48 h, and CP content was determined using the micro-Kjeldahl method (AOAC, 1990: method 976.05). Protein degradation rates were calculated using the first order kinetics model (Tamminga et al., 1994) (Table. 3.1). For the dietary inclusion levels DWP, the 11.2% level is based on an experiment by De Seram (2016) that showed that dietary inclusion of DWP up to 11.5% did not negatively affect productive performance of cows. Dietary treatments and chemical composition are presented in Table. 3.2. Diets were fed as

Table 3.1. In-situ rumen degradation characteristics of crude protein (CP) of soybean meal (SBM) and SoyPlus meal[1]

Items	SBM	SoyPlus
In-situ rumen degradation characteristics of CP[2]		
S (%)	8.33	9.76
D (%)	91.7	83.2
U (%)	0.00	7.01
T_0 (%)	1.09	2.47
K_d (%)	5.37	1.87
RUP (%)	48.6	70.7
EDCP (%)	51.4	29.3
EDCP (g/kg, DM)	236.8	127.2

[1]SoyPlus is a treated soybean product high in RUP (Landus Cooperative., Ames, Iowa).

[2]S = rapidly degradable fraction; D = potentially degradable fraction; U = undegradable fraction; T_0 = lag time (h), K_d = degradation rate of the potentially degradable fraction; and EDCP = effective degradability of CP.

Table 3.2. Ingredient and chemical composition of experimental diets

	Experimental Diets			
	0% DWP		11.2% DWP	
	9.5% RDP	11.5%RDP	9.5% RDP	11.5% RDP
Ingredient composition, % of diet DM				
Alfalfa hay	14.3	14.3	14.3	14.3
Barley silage	35.4	35.4	35.4	35.4
Barley grain	27.9	27.9	16.7	16.7
Pelleted ingredients[1]				
Dried whey permeate	0.00	0.00	11.2	11.2
Oat hulls	3.58	4.65	1.07	1.43
Soybean hulls	2.15	0.36	2.15	1.43
Soybean meal	1.07	12.5	4.29	15.0
SoyPlus[2]	11.4	0.72	10.7	0.36
Canola oil	0.61	0.61	0.61	0.61
Dairy premix[3]	1.80	1.80	1.80	1.80
Sodium bicarbonate	0.97	0.97	0.97	0.97
Salt	0.37	0.37	0.37	0.37
Limestone	0.23	0.23	0.23	0.23
Dynamate	0.17	0.17	0.17	0.17
Chemical composition				
DM, %	59.7	60.8	60.4	60.3
CP, % of DM	17.0	17.9	18.0	17.9
RDP, % of DM[4]	9.46	11.49	9.48	11.35
ADF, % of DM	22.0	21.3	20.3	20.3
NDF, % of DM	35.3	32.9	32.5	32.1
Starch, % of DM	24.4	22.9	18.0	17.6
Total WSC[5], % of DM	4.61	5.04	10.36	10.96
Ether extract, % of DM	2.21	2.58	2.50	2.70
Ash, % of DM	8.58	9.08	9.99	9.91
Calcium, % of DM	0.94	1.07	1.13	1.09
Phosphorus, % of DM	0.46	0.50	0.54	0.54
NFC[6], % of DM	36.9	37.6	37.1	37.4
NSC[7], % of DM	29.0	27.9	28.4	28.6
NE_L[8], Mcal/kg % of DM	1.49	1.52	1.51	1.52

[1]Ingredients were pelleted (pellet size: 4 mm) and then mixed in the appropriate proportions with steam-rolled barley to prepare the concentrate mixture at the Canadian Feed Research Centre.
[2]SoyPlus is a treated soybean product high in RUP (Landus Cooperative., Ames, Iowa).
[3]Dairy premix (Masterfeeds LP, Saskatoon, SK, Canada) contained (per kg of premix; DM basis): 250,000 IU of vitamin A; 80,000 IU of vitamin D3; 2,000 IU of vitamin E; 16%, Ca;

6.5% P; 6.3% Na; 7.0% Mg; 2,500 mg of Zn; 1,500 mg of Mn; 675 mg of Cu; 20 mg of Se; 80 mg of I; and 5.52% ground wheat as the premix carrier.

[4] RDP values based on diet formulations.

[5]Water-soluble carbohydrates, determined according to Hall et al. (1999), using lactose as a standard.

[6]Non-fiber carbohydrates= 100 - (%NDF + %CP + %ether extract + %ash).

[7]Non-structural carbohydrates= %WSC + %starch.

[8]Net energy of lactation; calculated from NRC (2001).

TMR, with a forage: concentrate ratio of ~50:50 (DM basis) (Table. 3.2). The forage component contained chopped alfalfa hay and barley silage, with a ratio of ~29:71 (DM basis).

3.3 Sample collection

Cows were fed daily at 0800 h and 1600 h. Daily feed intake and orts were recorded over the experimental period, and samples of TMR and orts were collected between day 20 and 23 and stored at -20°C. All cows were milked three times daily at 0400, 1200 and 1900 h, and milk production recorded. Milk samples were collected between day 20 and 22 and preserved with 2-bromo-2-nitropropane-1-2-diol. Milk samples were sent to CanWest DHI laboratory (Edmonton, AB) for compositional analysis.

On day 18 of each experimental period, ruminally-cannulated cows were fitted with a temporary vinyl catheter (0.86 mm I.D. × 1.32 mm O.D.; Scientific Commodities Inc., Lake Havasu City, AZ) in the left and right jugular veins. The catheters were used for collecting blood samples and isotope infusion. Between days 19 and 23, whole-body nitrogen balance, apparent total-tract nutrient digestibility, and urea-N kinetics were determined (Lobley et al., 2000). On day 19 of each experimental period, urine and fecal samples were collected to determine natural ^{15}N abundance. Double-labelled urea ([^{15}N, ^{15}N]-urea, 99.8 atom % ^{15}N; Cambridge Isotope Laboratories, Andover, MA) prepared in 0.15 M sterile saline was infused continuously through one jugular catheter starting at 1100 h on day 19 and ending at 0600 h on day 25 at a rate of 1-L/day/cow. The double-labelled urea dosage depended on the individual cow N intake (determined based on N intake between d 11 and 15 of the experimental period), such that the target was to achieve a urinary [^{15}N, ^{15}N]-urea enrichment of 0.15 atom percent excess. Between day 20 and 23 of each experimental period, fecal and urine samples were collected. Fecal samples were collected in metal trays positioned behind each tie-stall in the gutter and total daily fecal output was recorded. The feces were thoroughly mixed, and a 2.5% subsample was collected daily and stored at -20°C. Urine was collected and measured daily using a Bardex Foley indwelling bladder catheter (26 Fr, 75 cc ribbed balloon, lubricious-coated; C. R. Bard Inc., Covington, GA) which was inserted using the method described by Crutchfield (1968). Bladder catheters were inserted on day 18 and urine collection started at 1100 h on day 19 at the same time when isotope infusions were initiated. Urine was collected into 20-L carboy polythene containers and acidified to a pH of less than 3 by adding 150-mL concentrated HCl. Lowering urine pH prevents volatile NH_3 loss and

microbial degradation. A 50-mL sample was collected daily and stored at -20°C between day 20 and 23 and later analyzed for $[^{15}N, ^{15}N]$- and $[^{14}N, ^{15}N]$-urea enrichments. Also, a 5% subsample was collected every day and pooled per cow per period and stored at 20°C for later N analysis.

From day 20 to 23, ruminal pH was measured continuously at 1-min intervals using the Lethbridge Research Center Ruminal pH Measurement System (LRCpH; Dascor, Escondido, CA) as described by Penner et al. (2006) using the ruminally-cannulated cows.

For ammonia-N concentration (NH_3-N) and SCFA concentration analysis, a 1,000-mL ruminal digesta sample was collected by mixing 250-mL samples from the caudal ventral, cranial-ventral, cranial-dorsal and ventral rumen at 0900, 1500, 2100 on d 23, 0300, 1200, and 1800 on d 24, and 0000 and 0600 h on d 25, thus representing a 24-h feeding cycle.. The collected ruminal digesta samples were strained through 4 layers of cheesecloth, and two 10-mL aliquots were collected and mixed with 25% (w/v) meta-phosphoric acid (for SCFA analysis) or 1% H_2SO_4 acid (for NH_3-N analysis) before being stored at -20°C.

Blood samples were collected at the same time points as total collection. Blood samples were collected from the contralateral jugular vein into vacutainers with heparin and centrifuged at 4°C at 1,500 x *g* for 15 min. Plasma was stored at -20°C for later urea, glucose and BHBA analysis.

3.4 Sample analysis

After sample and data collection, frozen TMR, orts, and fecal samples were thawed overnight at room temperature. The DM content of TMR and orts samples was determined by drying in a forced-air oven at 50°C for 72 h, whereas fecal samples were dried at 50°C for 120 h (AOAC, 1990; method 930.15). Dried TMR and orts samples were ground through a 1-mm screen using a Christy-Norris mill (Christy and Norris Ltd., Chelmsford, England), and fecal samples were ground through a 1-mm screen on a Retsch ZM100 ultracentrifuge mill (Retsch-Allee 1-5, 42781 Haan, Germany). After grinding, the samples were pooled per cow per period. Before pooling, sub-samples of individual daily fecal samples were collected for ^{15}N enrichment analysis. Pooled TMR, orts, and fecal samples were sent to Cumberland Valley Analytical Services (North Middlesex, Ontario) for chemical analysis. Pooled samples were analyzed for ether extract (AOAC, 2006; method 2003.05), CP (AOAC, 2000; method 990.03), NDF (Van Soest et al., 1991 with modifications), ADF (AOAC, 2000; method 973.18 with modifications), starch (Hall, 2009),

and ash (AOAC, 2000; method 942.05). Total water-soluble carbohydrates (WSC) was analyzed using the method described by Hall, (2014) using lactose as a standard.

Daily composite milk samples were analyzed at CanWest DHI Laboratory (Edmonton, AB) for CP, fat, lactose and milk urea-nitrogen (MUN) using infrared spectroscopy (MilkoScan 605; Foss Electric, Hillerød, Denmark; AOAC, 1990; method 972.16).

Urine samples were thawed overnight at room temperature, and the micro-Kjeldahl method was then used to determine their N content (AOAC, 1990: method 976.05). Daily urine samples were analyzed for urea-N using the phenol-hypochlorite method (Fawcett and Scott, 1960) with absorbance set at 600 nm on a spectrophotometer (Model: 80-2097-62, Pharmacia LKB Biochrom, UK). Samples for [$^{15}N^{15}N$]- and [$^{14}N^{15}N$]-urea enrichment analysis were prepared by passing thawed daily urine samples containing 1.5 mg of urea through a pre-packed ion exchange column (Poly-Prep® Columns, AG® 50W-X8, hydrogen form #7316213; Bio-Rad, Richmond, CA) (Archibeque et al.,2001). After that, 7-mL of N-free water was passed through the columns with the eluate discarded, and urea was then eluted using 20-mL of N-free water. The eluate was collected into test tubes and then air-dried at 60°C and transferred into 17-x 60-mm borosilicate glass tubes using three 1-mL rinses with N-free water. The samples were then freeze-dried and were analyzed by isotope ratio mass spectrometry (IRMS; N-15 Analysis Laboratory, University of Illinois, Urbana-Champaign) for [$^{15}N^{15}N$-] and [$^{14}N^{15}N$-urea] enrichment. To account for non-monomolecular reactions, standards were prepared from $^{15}N^{15}N$-urea (99.8 atom % ^{15}N) and $^{14}N^{14}N$-urea (natural abundance urea; 0.364 atoms % ^{15}N) and analyzed, and the necessary corrections for $^{15}N^{14}N$-urea that is produced by non-monomolecular reactions were made (Lobley et al., 2000).

To determine NH_3-N concentrations, thawed ruminal fluid samples preserved with 1% sulphuric acid were vortexed and then centrifuged at $1,000 \times g$ for 10 min at 4°C. The supernatant was then analyzed for NH_3-N concentration using the method described by Fawcett and Scott (1960). For SCFA analysis, ruminal fluid samples preserved with metaphosphoric acid were centrifuged at $12,000 \times g$ at 4°C for 10 min. The resultant supernatant was then transferred to a microcentrifuge tube and centrifuged at $16,000 \times g$ for 10 min at 4°C. After that, 1-mL of the supernatant was mixed with 0.2-mL of isocaproic acid, which was used as an internal standard. The concentration of SCFA was measured using an Agilent GC system (Agilent 6890 Series, 254

Agilent Technologies, Waldbronn, Germany) with a column (30.0-m 47 × 320-μm × 0.25-μm; 255 model 7HM-G009-11, Zebron, Phenomenex, Torrance, CA). The column temperature was kept at 90°C for 0.1 min, then gradually increased to 170°C at a rate of 10°C/min. The column flow rate was 35-mL/min. The injector temperature was 170°C, and the detector temperature was 250°C (Khorasani et al., 1996).

To analyze for fecal N^{15} enrichment, daily fecal samples were pulverized using a ball mill grinder, and 2.4-mg of the sample was weighed into 8×12-mm tin capsules (Elemental Microanalysis Limited, Okehampton, UK) and put in a 96-well microtiter plate. After that, the samples were analyzed for ^{15}N enrichment by combustion to N_2 gas in a Costech ECS4010 elemental analyzer (Costech Analytical, Valencia, CA), and analyzed with continuous flow isotope ratio mass spectrometry (Delta V Advantage mass spectrometer, Thermo Scientific, Bremen, Germany).

Plasma urea-N concentration was measured using the phenol-hypochlorite method (Fawcett and Scott, 1960) with absorbance set at 600 nm on a spectrophotometer (Model: 80-2097-62, Pharmacia LKB Biochrom, UK). Plasma glucose concentration was measured using dianisidine dihydrochloride (No. F5803; Sigma) and glucose oxidase/peroxidase enzyme (No. P7119; Sigma, St. Louis, MO) and absorbance was measured using a plate reader at 450 nm (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA) (Bergmeyer et al., 1974). Plasma BHBA levels were measured in deproteinized plasma samples by oxidizing the BHBA to acetoacetate using 3-hydroxybutyrate dehydrogenase (H6501; Roche, Mississauga, Ontario, Canada) and reduction of NAD to NADH. After that, absorbance was measured using a plate reader at 450 nm (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA) (Williamson et al., 1962).

3.5   Calculations and statistical analysis

Ruminal pH was averaged for each minute, and the daily maximum, minimum, and mean pH reported. Thresholds of 5.8, 5.5 and 5.2 were used to determine the severity of ruminal acidosis. Duration and total area spent below each threshold was calculated. Total ruminal acidosis was classified as pH less than 5.8, mild ruminal acidosis was pH between 5.8 and 5.5, severe ruminal acidosis was pH between 5.5 and 5.2, and acute ruminal acidosis was pH below 5.2 (Penner et al., 2007).

Production data were analyzed using the Proc Mixed procedure of SAS, as a replicated 4 x 4 Latin square according to the following model: $Y_{ijklm} = \mu + S_i + P_j + C_{k(i)} + T_l + T_m + T_{lm} + ST_{il} + ST_{im} + E_{ijklm}$, where Y_{ijklm} is the dependent variable, μ is the overall mean, S_i is the fixed effect of square i, P_j is the fixed effect of period j, $C_{k(i)}$ is the random effect of cow k (within square i), T_l is the fixed effect of dietary treatment l, T_m is the fixed effect of diet m, T_{lm} is the interaction of treatment l and m, ST_{il} is the interaction between square i and treatment l, ST_{im} is the interaction between square i and treatment m and E_{ijklm} l is the residual error. Nitrogen balance, urea kinetics, total tract nutrient digestibility, and ruminal fermentation characteristics were analysed using the Proc Mixed procedure of SAS as a 4×4 Latin square according to the following model: $Y_{ijkl} = \mu + P_i + C_j + T_k + Tl + T_{kl} + E_{ijk}l$ where Y_{ijkl} is the dependent variable, μ is the overall mean, P_i is the fixed effect of period i, C_j is the random effect of cow j, T_k is the fixed effect of dietary treatment k, T_l is the fixed effect of dietary treatment m, T_{kl} is the interaction between treatment k and l, and E_{ijk} is the residual error. Treatment effects were declared significant when $P < 0.05$, and tendencies when $0.05 < P \leq 0.10$.

Data for maximum pH and area under pH 5.8 was not normally distributed, to fulfill the assumptions of the experimental design, maximum pH was transformed using logarithms, and for the area under pH 5.8, one outlier was eliminated.

4 RESULTS

4.1 Dietary characteristics

Diets were formulated to be isonitrogenous (~ 16.3% CP), but actual dietary CP content ranged from 17.0 to 18.0% (Table 3.2). Deviations in CP values from what was formulated is likely due to higher CP values in the final feed ingredients. As expected, the partial replacement of starch with 11.2% DWP reduced dietary starch content from 23.7% (range = 22.9 to 24.4%) to 17.8%. Also, as expected, diets with supplemental DWP contained more WSC (range = 10.4 to 11.0%) than those diets without supplemental WSC (range = 4.61 to 5.04%).

4.2 Production performance and plasma parameters

The dietary addition of DWP tended ($P = 0.09$) to increase DMI; however, actual and energy-corrected milk yields were not affected ($P > 0.05$) by the dietary addition of DWP or level of RDP (Table 4.1). Increasing dietary RDP levels had a tendency ($P = 0.08$) to decrease feed efficiency. Milk fat content and yield were not affected by diet ($P > 0.05$). The dietary addition of DWP increased milk protein content ($P = 0.01$) and yield ($P = 0.01$). Milk lactose content was similar in cows fed 9.5 and 11.5% RDP without added DWP, but milk lactose content was greater in cows fed 9.5% RDP compared to those fed 11.5% RDP with added DWP (interaction, $P < 0.01$), but dietary treatment had no effect on milk lactose yield ($P > 0.05$). Milk urea-N content was similar in cows fed 9.5 and 11.5% RDP with added DWP but was greater in cows fed 11.5% RDP compared to those fed 9.5% RDP without added DWP (interaction, $P = 0.03$). The addition of DWP decreased ($P = 0.01$) plasma urea-N concentration. Also, increasing dietary RDP level increased ($P = 0.02$) plasma urea-N concentration. Dietary treatment did not influence plasma glucose and BHBA concentrations ($P > 0.05$).

4.3 Apparent total-tract nutrient digestibility

Dietary addition of DWP had a tendency of increasing OM total-tract digestibility ($P = 0.10$; Table 4.2) but had no effect on DM total-tract digestibility ($P = 0.13$). There was no diet effect ($P > 0.05$) on total-tract digestibility of starch, NDF, and ADF. Total-tract CP digestibility was greater ($P = 0.02$) in cows fed 11.5% RDP compared to those fed 9.5% RDP. The dietary addition of DWP increased ($P = 0.04$) total-tract digestibility of ether extract. Total-tract digestibility of WSC was greater ($P < 0.01$) in cows fed the diet with DWP compared to those fed the diet without DWP.

Table 4.1. Feed intake, milk yield, and milk composition in dairy cows fed diets with or without dried whey permeate (DWP; as a partial replacement for barley grain) and with 9.5 or 11.5% (as % of dietary DM) ruminally-degradable protein (RDP) (n = 8).

| | Experimental diets | | | | | *P* value | | |
| | 0% DWP | | 11.2% DWP | | | | | |
Variable	9.5% RDP	11.5% RDP	9.5% RDP	11.5% RDP	SEM	DWP	RDP	DWP x RDP
DM intake, kg/d	29.0	29.1	29.7	30.6	1.19	0.09	0.29	0.37
Body weight, kg	769.5	760.0	771.1	773.3	15.4	0.26	0.57	0.38
Body weight change, kg/d	0.21	0.15	0.59	0.52	0.34	0.35	0.86	0.98
Milk yield, kg/d	36.2	36.0	36.5	36.6	2.00	0.47	0.95	0.79
ECM[1], kg/d	37.6	37.5	38.1	37.9	2.13	0.37	0.78	0.90
Feed efficiency[2]	1.32	1.27	1.31	1.26	0.04	0.77	0.08	0.87
Milk fat, %	3.69	3.73	3.86	3.69	0.15	0.54	0.57	0.36
Milk fat yield, kg/d	1.34	1.35	1.37	1.36	0.09	0.46	0.90	0.71
Milk protein, %	3.16	3.11	3.18	3.19	0.08	0.01	0.16	0.09
Milk protein yield, kg/d	1.12	1.12	1.17	1.22	0.05	0.01	0.41	0.40
Milk lactose, %	4.51[ab]	4.48[bc]	4.57[a]	4.41[c]	0.04	0.88	<0.01	<0.01
Milk lactose yield, kg/d	1.63	1.63	1.64	1.65	0.09	0.53	0.98	0.94
MUN[3], mg/dL	16.3[b]	19.0[a]	15.5[b]	15.7[b]	0.77	<0.01	0.02	0.03
Plasma urea-N, mg/dL	17.7	19.4	14.7	17.3	0.95	<0.01	0.02	0.49
Plasma glucose, mg/dL	53.6	55.8	53.6	56.3	1.80	0.88	0.18	0.88
Plasma BHBA[4], mg/dL	9.02	9.12	9.05	9.10	0.25	0.87	0.54	0.77

[1]Energy-corrected milk = [0.327 × milk yield (kg)] + [12.95 × fat yield (kg)] + [7.2 × protein   yield (kg)] (Orth, 1992).

[2]Feed efficiency = ECM/DM intake.

[3]Milk urea-N.

[4] Plasma ß-hydroxybutyrate, mg/dL.

Table 4.2. Apparent total tract digestibility in dairy cows fed diets with or without dried whey permeate (DWP; as a partial replacement for barley grain) and with 9.5 or 11.5% (as % of dietary DM) ruminally-degradable protein (RDP) (n = 4).

| Variable | Experimental diets | | | | SEM | *P* value | | |
| | 0% DWP | | 11.2% DWP | | | DWP | RDP | DWP x RDP |
	9.5% RDP	11.5% RDP	9.5% RDP	11.5% RDP				
Nutrient Digestibility, %								
DM	65.8	65.3	67.8	68.0	1.48	0.13	0.93	0.81
OM	66.8	66.8	68.9	69.7	1.40	0.10	0.78	0.78
Starch	86.5	83.7	85.9	87.8	2.42	0.49	0.86	0.35
CP	65.2	69.2	67.0	67.7	1.00	0.82	0.02	0.08
Ether extract	75.4	79.7	81.6	84.0	2.10	0.04	0.15	0.66
NDF	51.2	46.6	51.0	51.1	1.82	0.26	0.25	0.23
ADF	46.3	45.0	44.9	45.5	1.48	0.59	0.67	0.49
WSC[1]	87.0	87.6	94.0	95.1	0.77	<0.01	0.43	0.84

[1]WSC= water soluble carbohydrates.

4.4   Apparent nitrogen balance

Increasing dietary content of RDP ($P = 0.03$) increase DMI over the period when N balance was measured (Table. 4.3), while dietary addition of DWP ($P = 0.10$) tended to increase DMI. Increasing dietary content of RDP ($P = 0.08$) tended to increase N intake. Dietary addition of DWP also tended ($P = 0.08$) to increase N intake. Dietary addition of DWP increased urine excretion (kg/d) ($P = 0.03$), urinary N excretion (g/d) ($P = 0.01$), fecal N excretion (g/d) ($P = 0.03$), and milk N secretion (g/d) ($P < 0.01$). Increasing dietary RDP content increased urine excretion (kg/d) ($P = 0.05$), but it decreased fecal N excretion (% of N intake) ($P = 0.02$), total N excretion (% of N intake) ($P = 0.04$), and tended to decrease ($P = 0.08$) milk N secretion (% of N intake) compared to the low RDP diet. Dietary treatments had no effect ($P > 0.05$) on urea-N (g/d and as % urinary N), fecal excretion (DM, kg/d), and total N excretion (g/d). Apparent nitrogen balance was similar in cows fed 9.5 and 11.5% RDP with added DWP, but was higher in cows fed 11.5% RDP compared to those fed 9.5% RDP without added DWP (interaction, $P = 0.02$), and this same trend was observed with cow's productive N (interaction, $P = 0.04$).

4.5   Rumen fermentation characteristics

Dietary treatments did not affect ($P > 0.05$) mean ruminal pH, daily minimum pH, and area or duration when ruminal pH was less than 5.8 (Table. 4.4). Cows fed the low RDP diet had a greater maximum ruminal pH compared those fed the high RDP diet ($P = 0.03$). Dietary treatments did not affect ($P > 0.05$) ruminal concentrations of acetate, propionate, butyrate, and ammonia-N (Table 4.5). Dietary addition of DWP decreased ruminal concentrations of isobutyrate ($P = 0.02$), isovalerate ($P = 0.02$), total BCFA ($P = 0.02$), and total SCFA ($P = 0.03$). Increasing dietary RDP level increased ruminal concentration of isovalerate ($P = 0.03$), and total SCFA ($P < 0.01$). Increasing dietary RDP also tended to increase ruminal concentrations of valerate ($P = 0.08$) and total BCFA ($P = 0.06$). The acetate: propionate ratio tended to be greater in cows fed the low RDP diet compared to those fed the high RDP diet ($P = 0.06$).

4.6   Urea-N recycling kinetics

Dietary addition of DWP increased ($P < 0.01$) urea-N entry rate (UER) (Table. 4.6). Dietary increases in RDP also decreased ($P = 0.03$) UER. Urea-N gut entry rate (GER) tended to be greater ($P = 0.10$) in cows fed the low RDP diet compared to those fed the high RDP diet. There were no diet effects ($P > 0.05$) on urea-N returned to the ornithine cycle (ROC), urinary urea-N excretion (UUE), urea-N utilized for anabolism (UUA), and all fractional urea-N transfers ($P > 0.05$). The low RDP diet had higher ($P = 0.02$) urea-N loss to feces compared to the high RDP.

Table 4.3. N balance in dairy cows fed diets with or without dried whey permeate (DWP; as a partial replacement for barley grain) and with 9.5 or 11.5% (as % of dietary DM) ruminally-degradable protein (RDP) (n = 4).

| | Experimental diets | | | | | P value | | |
| | 0% DWP | | 11.2% DWP | | | | | |
Variable	9.5% RDP	11.5% RDP	9.5% RDP	11.5% RDP	SEM	DWP	RDP	DWP x RDP
DMI, kg/d	25.8	29.0	28.3	29.2	1.12	0.10	0.03	0.15
N intake, g/d	729.4	809.5	805.3	833.8	33.1	0.08	0.08	0.36
Urinary Excretion								
Total, kg/d	41.2	47.4	48.2	53.6	2.64	0.03	0.05	0.91
Total N, g/d	220.3	220.3	252.8	245.9	18.2	0.01	0.39	0.54
Total N, % of N intake	30.6	28.5	30.1	29.4	1.66	0.77	0.13	0.45
Urea-N, g/d	150.9	159.0	160.0	161.2	13.2	0.30	0.36	0.73
Urea-N, % urinary N	68.9	67.7	66.4	67.9	2.03	0.58	0.95	0.54
Fecal Excretion								
DM, kg/d	9.09	9.85	9.22	9.35	0.46	0.71	0.36	0.51
N, g/d	241.5	255.8	273.0	267.5	11.7	0.03	0.57	0.23
N, % of N intake	34.8	30.9	33.0	32.3	1.00	0.81	0.02	0.08
Total N Excretion								
g/d	462.9	483.9	522.4	520.0	31.4	0.16	0.77	0.72
% of N Intake	64.6	60.8	63.5	62.3	1.84	0.74	0.04	0.35
Milk N								
g/d	166.7	163.2	192.2	188.6	9.35	<0.01	0.28	0.98
% of N intake	24.1	21.0	22.4	22.3	0.84	0.83	0.08	0.11
Apparent N-balance, g/d	66.2[b]	163.6[a]	110.0[ab]	133.0[a]	19.3	0.57	<0.01	0.02
Productive N[1], g/d	237.8[b]	333.7[a]	293.0[ab]	318.8[a]	19.3	0.16	<0.01	0.04

[1]Calculated as N secreted in the milk + N apparently retained by the cow.

Table 4.4. Ruminal pH in dairy cows fed diets with or without dried whey permeate (DWP; as a partial replacement for barley grain) and with 9.5 or 11.5% (as % of dietary DM) ruminally-degradable protein (RDP) (n = 4).

| | Experimental diets | | | | | P value | | |
| | 0% DWP | | 11.2% DWP | | | | | |
Variable	9.5% RDP	11.5% RDP	9.5% RDP	11.5% RDP	SEM	DWP	RDP	DWP x RDP
Ruminal pH								
Daily mean	6.20	6.12	6.21	6.10	0.07	0.48	0.26	0.36
Daily maximum	6.55	6.53	6.56	6.49	0.12	0.11	0.03	0.19
Daily minimum	5.76	5.72	5.75	5.63	0.08	0.52	0.31	0.62
Duration pH < 5.8, min/d	45.4	71.2	131.4	121.5	66.0	0.34	0.91	0.79
Area pH < 5.8, pH × min/d	3.40	8.65	4.77	8.65	3.12	0.79	0.33	0.96

Table 4.5. Rumen fermentation characteristics in dairy cows fed diets with or without dried whey permeate (DWP; as a partial replacement for barley grain) and with 9.5 or 11.5% (as % of dietary DM) ruminally-degradable protein (RDP) (n = 4).

| | Experimental diets | | | | | P value | | |
| | 0% DWP | | 11.2% DWP | | | | | |
Variable	9.5% RDP	11.5% RDP	9.5% RDP	11.5% RDP	SEM	DWP	RDP	DWP x RDP
Ruminal SCFA[1], mmol/L								
Acetate,	75.8	73.1	71.8	71.2	1.88	0.29	0.45	0.49
Propionate	22.5	23.2	22.4	24.0	1.65	0.52	0.24	0.68
Isobutyrate	0.96	0.99	0.74	0.88	0.05	0.02	0.13	0.31
Butyrate	15.1	12.5	14.1	16.3	1.10	0.11	0.59	0.06
Isovalerate	1.43	1.59	1.12	1.40	0.10	0.02	0.03	0.44
Valerate	1.67	1.69	1.66	1.99	0.18	0.14	0.08	0.10
Total SCFA[1]	114.3[a]	117.0[a]	107.9[b]	116.5[a]	3.80	0.03	<0.01	0.05
Total BCFA[2]	2.39	2.58	1.85	2.28	0.15	0.02	0.06	0.44
Ammonia-N	12.8	15.0	12.2	14.1	0.96	0.52	0.12	0.88
Acetate: Propionate	3.49	3.16	3.27	3.08	0.17	0.15	0.06	0.57

[1]BCFA = branched-chain fatty acids.

[2]SCFA = short-chain fatty acids.

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Table 4.6. Whole-body urea-N kinetics in dairy cows fed diets with or without dried whey permeate (DWP; as a partial replacement for barley grain) and with 9.5 or 11.5% (as % of dietary DM) ruminally-degradable protein (RDP) (n = 4).

| | Experimental diets | | | | | P value | | |
| | 0% DWP | | 11.2% DWP | | | | | |
Variable	9.5% RDP	11.5% RDP	9.5% RDP	11.5% RDP	SEM	DWP	RDP	DWP x RDP
Urea-N kinetics[1], g/d								
UER	574.6	452.9	593.5	546.6	44.3	<0.01	0.03	0.07
GER	404.1	314.0	441.0	371.8	43.2	0.30	0.10	0.81
ROC	325.5	271.0	364.4	302.8	34.2	0.33	0.12	0.92
UUE	150.9	159.0	160.0	161.3	13.3	0.30	0.36	0.73
UFE	13.4	8.9	13.5	11.8	1.13	0.22	0.02	0.24
UUA	65.2	34.1	63.2	57.2	18.3	0.63	0.40	0.56
Fractional urea-N transfers								
UER to urine	0.27	0.36	0.28	0.31	0.03	0.76	0.14	0.46
UER to GIT	0.73	0.66	0.72	0.69	0.03	0.76	0.14	0.47
GER to ROC	0.82	0.89	0.84	0.81	0.04	0.48	0.62	0.29
GER to feces	0.03	0.03	0.03	0.03	0.004	0.88	0.81	0.61
GER to UUA	0.15	0.08	0.13	0.16	0.05	0.52	0.67	0.31

[1]UER = urea-N entry rate; GER = gastro-intestinal entry rate; ROC = return to ornithine cycle; UUE = urinary urea-N excretion; UFE = urea-N loss to feces; and UUA = urea-N utilized for anabolism.

5 DISCUSSION

5.1 Total-tract nutrient digestibility

One of the aims of this thesis research was to elucidate the impact of the partial replacement of barley grain with DWP, and its interaction with RDP on apparent total-tract digestibility in dairy cows. Improving total tract nutrient digestibility improves nutrient supply to the animal and may potentially lead to an increase in animal productivity. In this experiment partial replacement of barley grain with DWP had no effect on NDF digestibility, this is in agreement with the results observed by both Chibisa et al. (2015) who substituted corn or barley grain with DWP up to 6%, and De Seram (2016) who replaced barley grain with DWP up to 11.5%. In contrast, Vallimont et al. (2004) observed a quadratic increase in NDF digestibility when corn starch was replaced with sucrose up to 7.5% DM, in a dual-effluent continuous-culture system. Similary Broderick et al. (2008) observed a quadratic increase in NDF digestibility with increasing dietary sucrose content in dairy cows, but Khalili and Huhtanen (1991) observed a reduction in fibre digestion with supplementation of sucrose to cattle on grass silage.

The reasons for differences observed with NDF digestibility among these experiments that involved dietary supplementation with simple sugars could be related to ruminal pH and N availability. In the study by Khalili and Huhtanen (1991), sucrose was either infused continuously, dosed twice a day, or dosed twice a day with added sodium bicarbonate. Dietary addition of sucrose decreased NDF digestibility except for the treatment with added sodium bicarbonate. As discussed in section 2.6, sodium bicarbonate is a pH buffer, which would suggest that the reduction in NDF digestibility in that experiment was due to a decrease in ruminal pH. Low ruminal pH decreases NDF digestibility because it affects attachment of ruminal microbes to feed particles, and reduces the activity of some cellulolytic bacteria (McAllister et al., 1994). In the present study, ruminal pH was not affected by the dietary inclusion of DWP, so it can be surmised that the ruminal environment in cows fed DWP was optimum for fibre digestion. In fact, the mean ruminal pH for all treatments was above 6.0, which is the minimum threshold for optimum fibre digestion (Hoover, 1986).

Another factor that can potentially affect fibre digestion is ruminal N availability. Low ruminal N availability can reduce digestibility of fibre in the rumen (Russell, 1998; Tedeschi et al., 2000) and, because sugar utilizing microbes have a higher growth rate compared to cellulolytic

bacteria, the addition of sugar can lead to a reduction of ruminal NH_3 concentration which, in turn, can reduce the growth of cellulolytic bacteria. The retarded growth of cellulolytic bacteria can then result in reduced ruminal fibre digestion. Heldt et al. (1999) conducted two experiments, in which steers on a low-quality tallgrass-prairie hay diet were fed a diet supplemented with simple sugars with treatments being 0.031% of RDP (as % of BW) in the first experiment and 0.122% RDP (as % of BW) in the second experiment. In the first experiment NDF digestibility was lower in the diet with added simple sugars compared to the control, but in the second experiment, NDF digestibility was higher relative to the control when simple sugars were added to the diet. Heldt et al. (1999) suggested that sugar utilizing microbes have a faster growth rate than cellulolytic bacteria, and they use N faster, which depletes N for the slower-growing cellulolytic bacteria. Diets with high RFC but inadequate RDP are known to inhibit certain strains of cellulolytic bacteria like *fibrobacter succinogenes* (Russell, 1998). In the present study, all diets met the minimum RDP levels needed to maintain microbial function (NRC, 2001), and ruminal NH_3 concentration was above 5mg/dL (3.6 m*M*) which is the minimum threshold recommended by Schwab et al. (2005) to maintain optimum microbial growth. This could explain why there were no differences in fibre digestibility when dietary sugar and RDP contents were altered in the present study.

In this current study, WSC digestibility was increased by the dietary addition of DWP. The only research that I am aware of that reported the effect of simple sugars on WSC digestibility is De Seram (2016), and the results observed in that study are consistent with findings from the present study. Added WSC readily dissolve into the fluid phase of digesta, but WSC in the diet without added DWP are from the forage fraction of the diet and cell-bound, so for microbes to access these cell-bound WSC they first have to digest the cell wall (McAllister et al., 1994), this would reduce rate and extent of digestibility of these sugars. Additionally, the simple sugars present in forages, which are mainly pentoses, have a lower ruminal fermentation rate compared to the hexoses like lactose found in DWP (Emanuele and Sniffen, 2016). Dietary addition of DWP also led to a tendency for increased OM digestibility in the present study; these results are similar to the results reported by De Seram (2016) and is due to the partial replacement of starch with lactose which has a higher degradation rate (Van Amburgh et al., 2015)

Increasing RDP supply to the rumen also increases digestibility of nutrients. Valkeners et al. (2006) increased ruminal RDP supply by feeding diets with either 0g, 12.5g, or 33.3g of RDP

per kg of fermentable OM to bulls, which increased total-tract digestibility of OM and NDF. Gressley and Armentano (2007) reduced dietary RDP content by 28% from the predicted RDP requirements which led to a reduction in the apparent digestibility of OM, starch and fatty acids in lactating Holsteins. In this study, increasing dietary RDP content from 9.5 to 11.5% did not affect total tract digestibility for most nutrients, except for CP which increased with increasing dietary RDP content. Increasing RDP supply to the rumen usually leads to increases in ruminal NH_3 concentration, leading to increased microbial growth (Wickersham et al., 2009; Bailey et al., 2012), fermentation and ruminal feed degradation (Heldt et al., 1999; Valkeners et al., 2006). In this study, altering dietary RDP content had no impact on ruminal NH_3 content. The lack of response to dietary manipulation of RDP maybe because of variation between the actual RDP content in the experimental diets and the RDP content formulated for. The dietary RDP content indicated in Table 2.2 is derived from experimentally determined RDP content of soyplus and book values for RDP for all other dietary ingredients, actual RDP content of the experimental rations was not determined. The experimental diets were formulated to have a two-percentage unit deference, but actual RDP content may have been altered by variation between book values of ingredients and actual RDP values of ingredients, ingredient processing during manufacture, among other factors. The lack of response of ruminal NH_3 to RDP may help explain why there was no interaction between RDP and DWP on total tract nutrient digestibility.

Increasing synchrony between carbohydrates and N availability in the rumen has been shown to increases nutrient digestibility. Heldt et al. (1999) ran two experiments with steers on a low-quality tallgrass-prairie hay diet, in both experiments steers were supplemented either starch or simple sugars. In addition to the supplement simple sugars, steers were given a low RDP supplement in the first experiment and a high RDP supplement in the second experiment (0.031% vs 0.122% BW/d DM). Compared to the control (no added starch or simple sugars) NDF digestibility decreased in the first experiment, but in the second experiment, NDF and OM digestibility increased. This shows that the effect of simple sugars on nutrient digestibility is altered by N availability and increasing synchrony between carbohydrates and N availability in the rumen can potentially increase nutrient digestibility.

5.2 Ruminal fermentation characteristics

Partial replacement of barley grain with DWP did not affect ruminal NH_3 concentration. Results in past research involving inclusion of simple sugars on ruminal NH_3 concentration have been variable, with ruminal NH_3 concentration either increasing (Oba et al., 2015), decreasing (Chibisa et al., 2015; De Seram, 2016), or not changing (DeFrain et al., 2004; Vallimont et al., 2004; Penner et al., 2009b). Ruminal NH_3 concentration is dependent on diet fermentability (Abdoun et al., 2006). DeFrain et al. (2006) conducted an experiment that demonstrates that dietary addition of simple sugars reduces ruminal NH_3 concentration in diets where MPS is limited by diet fermentability. DeFrain et al. (2006) added lactose (15.7% DM) to pre and post-partum cow rations, adding lactose to the pre-partum ration which was high in forage, decreased ruminal NH_3 concentration, but the addition of lactose to the post-partum diet which was high in concentrates had no effect on ruminal NH_3 concentration. Another potential reason why partial replacement of barley grain with DWP in my experiment did not affect ruminal NH_3 concentration is because of microbial glycogen accumulation. Addition of simple sugars to ruminant rations has been shown to increase microbial glycogen accumulation (Lou et al., 1997a; Hall, 2011), this would reduce diet fermentability and incorporation of NH_3-N into microbial protein. Reduced diet fermentability because of microbial glycogen accumulation could also help explain why partial replacement of barley grain with DWP in my experiment decreased total ruminal SCFA concentration.

Dietary manipulation of RDP also had no effect on ruminal NH_3 concentration in my experiment, this was against expectations since increasing dietary RDP content always lead to increased ruminal concentration of NH_3 (Wickersham et al., 2009; Bailey et al., 2012; Wang et al., 2012; Mutsvangwa et al., 2016). The lack of response to altering RDP content may be because the difference in RDP content between diets may not have been large enough to elicit changes in NH_3 concentration. The dietary RDP content reported in Table 2.2 is derived from experimentally determined RDP content of soyplus and book values for RDP for all other dietary ingredients, actual RDP content of the rations was not determined. Actual RDP content of the experimental diet may have varied from the formulated RDP content because of among various factors ingredient processing during feed manufacture, and variation between book values of ingredients and actual RDP values.

Altering dietary RDP content also had no effect on ruminal profiles of the major VFA (i.e., acetate, propionate, and butyrate). The results observed in my experiment are in line with results observed in experiments with lactating dairy cows fed diets with varying RDP content (Gressley and Armentano, 2007; Broderick and Reynal, 2009; Mutsvangwa et al., 2016). However, experiments with diets high in forages do not show the trend observed in my experiment. Wickersham et al. (2009) ruminally dosed steers on a low-quality prairie hay diet, with casein at rates of 0, 59, 118, or 177 mg of N/kg of BW daily, leading to reduced ruminal concentration of acetate and butyrate, and increased ruminal propionate concentration. Bailey et al. (2012) also ruminally dosed steers on a low-quality prairie hay diet, with casein at rates of 120 or 240g daily and this increased ruminal concentration of acetate and propionate. The variation between experiments using high forage and high concentrate diets is probably due to variations in diet fermentability and RDP concentration.

Even though changing RDP content from 9.5 to 11.5% did not affect the major VFA concentrations, it increased the ruminal concentration of isovalerate, and tended to increase ruminal concentrations of valerate and total BCFA. Increases in ruminal concentrations of individual and total BCFA were expected with increased dietary RDP content as BCFA are a by-product of ruminal protein degradation (Liu et al., 2018); therefore, increasing the proportion of dietary protein degraded in the rumen (i.e., RDP) would be expected to increase ruminal production of BCFA. Results similar to mine have been observed in experiments with both steers (Wickersham et al., 2009; Bailey et al., 2012) and dairy cows (Broderick and Reynal, 2009; Mutsvangwa et al., 2016).

Increasing dietary RDP content increased total ruminal SCFA concentration in this experiment. Increasing the CP content or the proportion of CP that is ruminally degradable (RDP) in rations that are not deficient in RFC, increases the supply of N (NH_3, free AA and peptides) to microbes leading to increased MPS, increasing ruminal microbial activity and this leads to an increase in VFA production in the rumen (Satter and Slyter, 1974; Pilachai et al., 2012). In the experiments done by Bailey et al. (2012) and Wickersham et al. (2009), increasing RDP supply to the rumen increased ruminal concentrations of NH_3 and total ruminal concentrations of VFA. In my experiment manipulation of dietary RDP did not affect ruminal NH_3 concentration, and it is unclear why increasing RDP content in my experiment, increased total SCFA concentration.

Typically in high concentrate dairy experiments increasing dietary RDP content does not increase ruminal concentration of SCFA (Reynal and Broderick, 2005; Broderick and Reynal, 2009; Mutsvangwa et al., 2016) because ruminal concentrations of NH_3 are usually above the threshold of 5mg/dL (3.6 mM) recommended by Schwab et al. (2005). Increasing ruminal supply of RDP in animals with ruminal NH_3 concentrations less than 5mg/dL (3.6 mM), increases the total ruminal concentration of VFA (Wickersham et al., 2008; Bailey et al., 2012). Schwab et al. (2005) recommends concentrations between 5 and 11 mM depending on diet fermentability, diets with higher fermentation rates need higher NH_3 concentration to maintain MPS, and Odle and Schaefer (1987) recommends ruminal NH_3 concentrations of 8.9 mM for ground barley.

Dietary inclusion of DWP did not affect ruminal concentrations of butyrate, propionate, and acetate. Reports in the literature indicate that the addition of simple sugars to ruminant diets does not always lead to a change in the ruminal concentration of acetate and propionate (DeFrain et al., 2006; Penner et al., 2009b; Chibisa et al., 2015; De Seram, 2016); however, unlike the results that were observed in the present study, dietary inclusion of lactose typically results in increased ruminal concentrations of butyrate (DeFrain et al., 2004, 2006; Chibisa et al., 2015; De Seram, 2016). The reasons why providing supplemental sugar did not alter ruminal butyrate concentration in the present study are not clear; however, it should be noted that mean ruminal concentration was numerically greater (15.2 vs 13.8 mmol/L) in cows fed DWP with a P value = 0.11; therefore the lack of a significant effect could be reflective of the small sample size ($n = 4$) in the present study. Also, the ruminal concentration of VFA is a product of production versus removal, and the lack of response due to the provision of supplemental sugar may be due to increased butyrate absorption out of the rumen since it has the greatest absorption rates (compared to the other major VFA) because of its high lipophilicity (Aschenbach et al., 2011). However, the concentration of BHBA also was not altered making it unlikely that the lack of response observed is due to increased absorption rates of butyrate.

Concentrations of the branched-chain VFA, isobutyrate, and isovalerate decreased with the dietary addition of DWP which led to a reduction in total BCFA in the rumen. The results observed in the current study concur with past studies (DeFrain et al., 2004, 2006; Chibisa et al., 2015; De Seram, 2016). As already discussed above, BCFA are a by-product of protein degradation, namely isobutyrate and isovalerate are by-products of degradation of valine and leucine, respectively

(Allison, 1978). When the supply of readily-fermentable energy is limiting as might be the case with diets not containing DWP in the present study, ruminal microbes breakdown AA to provide ATP for their growth and maintenance (Russell et al., 1983). Therefore, the reduction in the ruminal concentration of BCFA might be indicative of reduced reliance of ruminal microbes on protein as a source of energy in diets containing DWP

Increasing dietary sugar content does not usually affect total ruminal VFA concentration (Vallimont et al., 2004; Penner and Oba, 2009; Chibisa et al., 2015), but in the present study total VFA concentration was reduced by the dietary addition of DWP despite the tendency for increased total tract OM digestibility and DMI. Since the ruminal concentration of SCFA is a product of production versus removal, there may have been an increase in SCFA removal from the rumen. Chibisa et al., (2015) recorded increased Cl^- competitive absorption of acetate and propionate when grain starch was partially replaced by DWP. Volatile fatty acid removal from the rumen was not measured in this study. A more plausible explanation as to why there was a reduction in total SCFA concentration with supplemental DWP could be partly related to increased bacterial sequestration of dietary carbon into microbial protein or glycogen. Increased sequestration of dietary C into amino acids and glycogen decreases substrates for VFA production. Simple sugars have in the past been observed to increase MPS (Kim et al., 1999; Bailey et al., 2012) and, according to the Cornell Net Carbohydrate and Protein System, sugar-utilizing bacteria produce at least 18% more microbial protein than bacteria utilizing other carbohydrate fractions. Ruminal microbes also increase glycogen synthesis in the presence of simple sugars. In a study by Lou et al. (1997), *Prevotella ruminicola*, which is a ruminal microbe that can constitute 19% of the ruminal microbial population, was incubated together with maltose and results indicated that 40% of the maltose was used by the microbes to synthesize glycogen. Protozoa also synthesize glycogen, and this synthesis is increased in the presence of simple sugars (Hall, 2011). The storage of simple sugars as glycogen in ruminal bacteria and protozoa shields those simple sugars from ruminal fermentation, thus potentially reducing SCFA production in the rumen.

5.3   Ruminal pH

One of the goals of dairy cow nutrition is to increase milk production up to the genetic potential of the cow by satisfying the cow's energetic needs by increasing diet fermentability. Simple sugars have a higher fermentation rate compared to other carbohydrate fractions (Van

Amburgh et al., 2015), and a major concern when feeding simple sugars to ruminants is that animals may develop ruminal acidosis. Past studies have shown that even though simple sugars have higher fermentation rates than grain starch (Van Amburgh et al., 2015), partial replacement of grain starch with simple sugars does not always lead to a decrease in ruminal pH (DeFrain et al., 2004; Vallimont et al., 2004; Chibisa et al., 2015). In fact, in some studies the partial replacement of grain starch with simple sugars resulted in an increase in ruminal pH (Penner and Oba, 2009), suggesting that simple sugars can be used to increase overall animal productivity without increasing the animals' susceptibility to ruminal acidosis.

One of the primary objectives of this study was to investigate the effect of partial replacement of barley grain with lactose in the form of DWP on ruminal pH. In this study, partial replacement of barley grain with DWP up to 11.2% did not affect min, max, mean pH, and time and area spent under the pH threshold of 5.8. These results are in agreement with past studies that have investigated the dietary inclusion of lactose as DWP, or in its pure form. DeFrain et al. (2004) experimented with lactating dairy cows fed either low lactose, high lactose, or liquid whey (7.1%, 14.3%, or 9.4% of diet DM, respectively) as a partial replacement for corn grain. Results from that study indicated that the dietary substitution of corn grain with lactose or liquid whey did not influence ruminal pH. Similar results were observed by Chibisa et al. (2015), who partially replaced corn or barley grain starch with lactose in the form of DWP up to 6% (7.9% TS), and De Seram (2016) when he partially replaced barley grain with DWP up to 11.5% (12.6% TS).

It is not clear why partial replacement of grain starch with simple sugars does not lead to a reduction in ruminal pH. Changes in VFA profiles associated with increasing diet ruminal fermentability may increase VFA absorption from the rumen leading to a stabilization in ruminal pH (Gäbel et al., 1991). Increasing dietary sugar content usually leads to increased butyrate production (Heldt et al., 1999; DeFrain et al., 2006; Chibisa et al., 2015). Butyrate is known to stimulate growth and proliferation of ruminal epithelia (Tan and Murphy, 2004) through the effects of IGF-1 and EGF (Penner et al., 2014). There is also evidence that shows an increase in the expression of ruminal epithelial transport proteins. Chibisa et al. (2015) observed an increase in chloride competitive absorption rates of propionate and acetate when DWP partially replaced grain starch. Chloride competes with SCFA for the SCFA$^-$/HCO$_3$ anion exchange pathway, and at rumen concentration of 40 mM, chloride inhibits absorption of propionate and acetate (Aschenbach

et al., 2009), normal rumen chloride concentration is typically in the range of 16 to 20 m*M* (Duffield et al., 2004; Shen et al., 2012) . Chloride competitive absorption rates of VFA is deduced by measuring WRR disappearance rates of VFA using a high chloride buffer with chloride concentrations above 40 m*M* and a low chloride buffer, and the difference is Chloride competitive absorption. Increased VFA removal because of the increased ruminal epithelial surface area and epithelial transport proteins may explain why ruminal pH does not decrease on high sugar diets. In the current study, concentrations of ruminal butyrate and plasma BHBA (which is an indirect indicator of ruminal butyrate concentration as it is primarily derived from the ruminal epithelial metabolism of butyrate absorbed from the rumen) were not affected by the dietary inclusion of DWP. Therefore, it is unclear if the mechanisms described above (i.e., increased ruminal epithelial surface area and expression of epithelial transport proteins) were up-regulated in cows fed DWP. However, it should be noted that the ruminal and plasma BHBA concentrations are a snapshot in time and do not necessarily reflect the rates of production of these metabolites.

Another potential reason why ruminal pH was not depressed by the dietary addition of DWP is that the carbon skeleton derived from dietary sugar that would otherwise be used for VFA formation may have been used instead for de-novo synthesis of amino acids together with NH_3, or the supplemental sugar could have been diverted towards the formation of microbial storage carbohydrates instead of VFA production. As discussed in previous sections, increasing dietary sugar content (Kim et al., 1999; Bailey et al., 2012) increases MPS, and sugar fermenters produce 18% more microbial protein. In this study, we did not measure MPS, but there was an increase in milk protein yield suggesting increased MPS.

As discussed previously, ruminal bacteria and protozoa are known to convert carbohydrates into glycogen for short-term storage (Hall and Weimer, 2007). It has been demonstrated that in the presence of simple sugars, ruminal bacteria and protozoa (Lou et al., 1997; Hall, 2011) increase glycogen synthesis. As ruminal microbes flow out of the rumen, this "protects" the simple sugars from ruminal fermentation, thus potentially reducing ruminal production of SCFA.

5.4   Production data

One of the primary objectives of this study was to investigate the interactive effects of increasing diet fermentability by partially replacing barley grain with DWP up to 11.2% DM and

dietary RDP content (9.5% vs 11.5% DM) on DMI, milk yield, and milk components. Partial replacement of barley grain with DWP tended to increase DMI. Dry matter intake in dairy cows is influenced by rumen distention among various other factors; therefore factors that increase passage rate like digestion rate can improve DMI (Allen, 2000). Simple sugars have a higher fermentation rate compared to other carbohydrate fractions (Weisbjerg et al., 1998), which increases bacterial activity and feed degradation rates. In this study, partial substitution of barley grain with DWP tended to increased OM digestibility, which may have contributed to the increased DMI observed with the dietary inclusion of DWP. Additionally, DMI in ruminants is also influenced by palatability, as there is experimental evidence that shows that DMI is increased when sheep, goats, and cattle are fed hay harvested later in the day as compared to the morning (Fisher et al., 1999, 2002). Sugar content in forages peaks during the afternoon when photosynthesis is at its highest (Owens et al., 1999), and Broderick and Radloff (2004) suggested that sugar potentially increases DMI by increasing diet sweetness. However, the effects of dietary inclusion of simple sugars on DMI have not been consistent in past studies. Similar results to those observed in the present study have been reported by Broderick and Radloff (2004), who partially replaced high-moisture shelled corn with either dried or liquid molasses up to 12% DM and reported that dried molasses linearly increased DMI, whereas DMI responded quadratically to liquid molasses. Similarly, Broderick et al. (2008) partially replaced high-moisture shelled corn with sucrose up to 7.5% DM, and observed that there was a linear increase in DMI. However, when Chibisa et al. (2015) substituted either corn grain or barley grain with DWP (up to 6% DM), DMI was not affected. De Seram (2016) also partially replaced barley grain with DWP up to 11.5% DM without affecting DMI. The reasons for the variation in the effects of simple sugars on DMI is not well understood (Penner, 2015), but could be related to differences in basal dietary composition, the type of cereal grain being replaced by simple sugars, and the level of DMI of experimental animals used.

Dietary RDP content had no effect on DMI in this current experiment. According to the NRC (2001), there is a positive correlation between dietary RDP content and DMI, and increasing dietary RDP content by two percentage units is predicted to increase DMI by 1.1 kg/d. The positive effects of increased dietary RDP intake is attributed to increased supply of ruminally available N (AA, NH_3, and peptides) leading to increased microbial growth (Wickersham et al., 2008; Bailey et al., 2012), and ruminal feed degradation (Heldt et al., 1999; Valkeners et al., 2006).

However, as observed in the present experiment, increasing dietary RDP content does not always lead to increased DMI. Bahrami-Yekdangi et al. (2016) fed a diet with either 10.9, 10.0 or 9.3% RDP content (DM basis) to lactating Holstein cows and observed that there was no effect on DMI. Also, Mutsvangwa et al. (2016) fed either a low or high RDP diet (63% vs. 69%, as % of CP), and reported that dietary RDP content had no effect on DMI. Similarly, in another experiment by Tacoma et al. (2017), increasing dietary RDP content had no effect on DMI. In contrast, in an experiment by Cyriac et al. (2008) in lactating dairy cows fed diets containing either 11.3, 10.1, 8.8, or 7.6% RDP, the results showed a reduction in DMI in the cows on the 7.6% RDP diet. Cyriac et al. (2008) then suggested that the NRC (2001) overstates the requirements for dietary RDP content, and diets with RDP contents as low as 8.8% RDP supply adequate N (based on ruminal NH_3 concentration) to satisfy microbial N requirements. In the present study, all diets met the minimum RDP levels needed to maintain microbial function (NRC, 2001); across diets, ruminal NH_3 concentrations were well above 5 mg/dL, which is the minimum threshold recommended by Schwab et al. (2005) to maintain optimum microbial growth.

Despite the tendency for increased DMI and OM total-tract digestibility when barley grain was partially replaced with DWP in the present study, milk yield and ECM were not affected. Feed digestibility and DMI have a major impact on milk production, as increasing DMI and nutrient digestibility increases post-ruminal nutrient supply for milk production. Results from past studies have been mixed, and dietary addition of simple sugars has not always translated into increased milk output. Similar results to those reported in the present study were observed by Chibisa et al. (2015) when lactating Holstein cows were fed a barley or corn-based diet with 6% DWP, and De Seram (2016) who replaced barley grain with DWP up to 11.5% DM. In both experiments, DMI was not impacted by DWP inclusion in the diet, which partly explains the results they observed. In contrast, Broderick and Radloff (2004) observed a quadratic increase in both DMI and milk production when dietary sugar content was increased using molasses. The reason why milk yield was not reflective of DMI and feed digestibility in the present experiment is unclear. A potential reason that may partly explain why the tendency for increased DMI and OM digestibility did not translate into increased milk yield is because of increased energy spilling reactions when the supply of simple sugars is increased. Energy spilling refers to reactions where energy is used for cell processes that do not contribute to cell growth (Strobel and Russell, 1986). An example of a process that utilizes energy but does not contribute to growth is the synthesis of storage

carbohydrates. Ruminal microbes are known to synthesize glycogen (a storage carbohydrate) in the presence of simple sugars, and glycogen synthesis is an energy-consuming process that contributes to energy spilling (Lou et al., 1997b; Hall, 2011). Diversion of energy towards energy spilling deprives the microbes (and host animal) of energy for productive functions and could be partly responsible for the lack of response in milk production when barley grain was partially replaced with DWP in this experiment.

Total milk yield and ECM did not change when dietary RDP content was increased in the current experiment. According to the NRC (2001), increasing dietary RDP content by two percentage units should increase daily milk yield by 2 kg. However, the benefits of increased RDP content to milk yield is seldom observed in dairy cows. Studies by Bahrami-Yekdangi et al. (2016), Mutsvangwa et al. (2016), and Tacoma et al. (2017) which involved altering dietary RDP content showed no change in milk yield. In an experiment by Cyriac et al. (2008) where lactating dairy cows were fed diets with RDP contents ranging between 11.3 and 7.6% DM, only a tendency for reduced milk yield at the lowest RDP content was observed. The observations from that study support the assertion by other researchers (Cyriac et al., 2008; Agle et al., 2010; Chibisa, 2013) that the NRC (2001) overstates RDP requirements for dairy cows and 8.8% is adequate to maintain animal productivity. It had been anticipated that increasing dietary RDP content would increase ruminal NH_3 concentration, leading to increased microbial growth and activity, ruminal feed degradability, which would increase post-ruminal nutrient supply for milk production. However, in this experiment NH_3 content, OM digestibility and DMI were not affected by the dietary addition of RDP, so it was not surprising that milk yield was not influenced by dietary RDP content.

In agreement with previous studies (DeFrain et al., 2004; Penner et al., 2009b; Chibisa et al., 2015; De Seram, 2016) that have investigated the effects of increasing dietary sugar content on production, the partial replacement of barley grain with DWP had no effect on milk fat content and yield in the present study. However, increasing dietary sugar content sometimes leads to increased milk fat yield. Penner and Oba (2009) fed dairy cows diets with either high or low sugar contents (8.4% vs 4.7% DM) and observed a tendency for an increase in milk fat yield with increased dietary sugar content. Broderick et al. (2008) also observed a quadratic increase in milk fat yield with increasing dried molasses inclusion in the diet. The increase in milk fat yield which is sometimes observed with increased dietary sugar content has largely been attributed to changes

in ruminal butyrate (Khalili and Huhtanen, 1991) and acetate concentrations, and plasma BHBA concentration (a product of epithelial butyrate metabolism), which are precursors for mammary de-novo milk fatty acid synthesis (Palmquist et al., 1969). In this experiment, concentrations of butyrate and acetate in the rumen, and plasma BHBA concentration were not affected by dietary inclusion of DWP, which explains why milk content and yield was not affected by dietary inclusion of DWP.

Increasing dietary RDP content from 9.5% to 11.5% of DM had no effect on milk fat content and yield. Similarly, Reynal and Broderick (2005) increased dietary RDP content from 10.6% to 13.2% of DM without affecting milk fat content and yield. Tacoma et al. (2017) also fed a high RDP: RUP diet compared to a low RDP: RUP diet (62:38 vs 51:49 as a proportion of CP) and reported that there was no change in milk fat content. In contrast, Cyriac et al. (2008) did not observe a change in milk fat content when dietary RDP content was increased from 7.6% to 11.3% DM, but observed a linear increase in milk fat yield with increasing dietary RDP content. Likewise when Savari et al. (2017) fed either a high or low RDP: RUP ratio (65:35 vs 60:40 as a % of CP), increasing dietary RDP content increased milk fat yield without affecting milk fat content. In the current experiment, the lack of response to changes in RDP content in terms of fat content and yield is because in this experiment dietary RDP content had no effect on ruminal acetate, butyrate, and plasma BHBA concentration, which as explained in previous sections has an impact on milk fat composition and yield.

In this experiment, the partial substitution of barley grain with DWP increased milk protein content and yield. In agreement with the current experiment, Broderick and Radloff (2004) partially replaced high-moisture shelled corn with either dried or liquid molasses and observed that the dietary addition of liquid molasses linearly increased milk protein content and had a quadratic effect on milk protein yield, whereas dried molasses had no effect on milk protein content but had a cubic effect on milk protein yield. However, in most experiments that have investigated the effect of simple sugars on animal productivity, simple sugar addition to the diet has had no effect on milk protein content or yield (DeFrain et al., 2004; Chibisa et al., 2015; De Seram, 2016). Sannes et al. (2002) suggested that milk protein production is dependent on the diet's ability to stimulate microbial protein synthesis. Increasing the supply of ruminally-fermentable carbohydrates is known to increase microbial protein synthesis (Theurer et al., 1999),

leading to increases in post ruminal supply of metabolizable protein for milk protein synthesis (Martineau et al., 2011). This seems to suggest that the increased milk protein production in this experiment may be due to improved AA supply because of increased MPS. However, De Seram (2016) substituted 11.5% DM of barley grain with DWP to lactating cows and observed that there was no change in milk protein content even though there was a quadratic increase in total microbial non-ammonia N flow.

Increasing the proportion of RDP in the diet from 9.5 to 11.5% DM in this experiment had no effect on milk protein content or yield. Increasing dietary RDP content is known to increase DMI and availability of N substrates (AA and NH_3) for MPS (Wickersham et al., 2008; Bailey et al., 2012). Increasing MPS increases the post-ruminal supply of high-quality protein (in terms of AA constitution and digestibility) which increases milk protein yield. In support of this, Reynal and Broderick. (2005) fed dairy cows diets that ranged from 10.6 to 13.2% RDP (% of DM) and observed linear increases in milk protein content and quadratic increases in milk protein yield. However, increasing dietary RDP content usually has no effect on milk protein content or yield. Mutsvangwa et al. (2016) fed either a low or high RDP diet to lactating Holstein cows and reported that dietary RDP content had no effect on milk protein content or yield. Similar results were observed by Tacoma et al. (2017), Armentano et al. (1993), and Gressley and Armentano (2007). Santos et al. (1998) suggested that the reduction in MP synthesis that can potentially happen with a reduction in RDP supply to the rumen is offset by an increase in RUP flow which maintains metabolizable protein supply to the animal, thereby maintaining milk protein yield. In this experiment the diets were isonitrogenous; therefore, a reduction in dietary RDP content was coupled with an increase in RUP and this may have potentially offset the reduction in milk protein yield when dietary RDP content was reduced.

Milk lactose content was greater in cows fed the low RDP diet compared to those fed the high RDP diet with added DWP, but altering RDP content had no effect on the diet without added DWP (interaction); however, dietary treatment had no effect on milk lactose yield. Lactose has an osmoregulatory role in milk and is responsible for drawing water into the mammary epithelial cells (Jenkins and McGuire, 2006). For this reason, milk lactose content is fairly constant, and can only be changed under extreme conditions. It is not clear why lactose content changed in this experiment as it is not easily manipulated by dietary changes (Jenkins and McGuire, 2006).

Partial substitution of barley grain with DWP in this experiment decreased plasma urea-N from 18.6 mg/dL to 16.0 mg/dL. The urea present in plasma is synthesized in the liver from NH_3 mainly derived from the rumen (Storm et al., 2012). Plasma urea-N is correlated with urinary urea-N excretion, which makes it a good indicator of N efficiency (Ruska and Jonkus, 2014) and N balance (Hammond, 1997). In this experiment, ruminal NH_3 concentration and urinary urea-N production did not change, while endogenous urea-N increased when DWP was added to the diet. It is not clear why plasma urea-N decreased in this experiment when DWP was added when there was no change in rumen NH_3 concentration and there was an increase in endogenous urea-N production.

Increasing dietary RDP content from 9.5% to 11.5% DM increased plasma urea-N concentration by 12% from 16.2 mg/dL to 18.4 mg/dL in this experiment. The results in this experiment are in agreement with most experiments where dietary RDP content was altered, which have reported that increasing dietary RDP content invariably leads to increased plasma urea-N (Histrov, 2006; Tacoma et al., 2017) or blood urea-N (Reynal and Broderick, 2005; Gressley and Armentano, 2007; Savari et al., 2017). This increase is fueled by increased ruminal NH_3 concentrations and UER (Wickersham et al., 2008). However, in this experiment, there was only a slight numerical increase in ruminal NH_3 concentrations, and UER decreased during this experiment when dietary RDP content was increased. It is not clear why plasma urea-N concentrations increased while UER decreased. The increase in plasma urea-N may be because fractional transfer rates of UER into the GIT showed a numerical decrease which may have led to an accumulation of urea-N in the plasma.

Another important indicator of N efficiency is milk urea-N concentration. Milk urea-N concentration is positively correlated with urinary urea-N excretion and can be used to calculate urea excretion (Jonker et al., 1998). In the current experiment, milk urea-N was higher in cows fed the high RDP diet without added DWP when compared to cows fed the low RDP diet without added DWP (interaction). Milk urea-N is derived from circulating blood urea-N which freely diffuses between mammary glands and blood until an equilibrium is reached; therefore, changes in MUN concentration follows that of BUM (Jonker et al., 1998). However, in this experiment, the changes that were observed in plasma urea-N concentration, milk urea-N concentration, and urinary urea-N excretion were not parallel, and the reasons for that are not clear.

Partial replacement of barley grain with DWP had no effect on plasma BHBA concentrations in this experiment. An increase in plasma BHBA concentration is often associated with increased dietary sugar content (DeFrain et al., 2004, 2006; Penner and Oba, 2009). This increase in plasma BHBA concentration is because of increased ruminal butyrate production. Plasma BHBA is primarily a product of metabolism of butyrate by ruminal epithelial cells (Van Houtert, 1993), and increased ruminal butyrate concentration leads to increased BHBA production by ruminal epithelial cells. In the current experiment, ruminal butyrate concentration was not affected by the partial replacement of barley grain with DWP, which should partly explain the lack of response of plasma BHBA concentration to increased dietary sugar content.

5.5 Nitrogen balance

The efficiency of conversion of dietary N to milk N is low in dairy cows, and up to 80% of dietary N can be lost in urine and feces (Mulligan et al., 2004). Because protein sources are relatively expensive compared to other feed ingredients, the poor efficiency of N utilization represents financial losses to dairy producers. Additionally, N excretion particularly urinary urea-N is responsible for land, water, and air pollution (Varel et al., 1999). Nitrogen utilization can be improved by altering the proportion of dietary N that is degraded in the rumen (RDP) or increasing diet fermentability. One of the primary goals of this study was to investigate the effects of increasing diet fermentability by the partial replacement of barley grain with DWP on N utilization in dairy cows, and the interaction between dietary inclusion of DWP and dietary RDP content.

Increasing dietary RDP content from 9.5 to 11.5% of dietary DM and the partial replacement of barley grain with DWP both tended to increase N intake in the present study. Because diets differing in sugar (or RDP contents) varied marginally in CP content, the tendencies for differences in N intake due to dietary addition of DWP or RDP content in this experiment were largely due to differences in DMI that were observed during the 4-d total collections.

Dietary addition of DWP increased urine volume in the current experiment. The volume of urine is affected mainly by the total water intake and the quantity of solutes that need to be excreted (N, Na, and K in particular) (Bannink et al., 2010). In this experiment, water intake was not measured; however, water intake is known to have a positive correlation with nitrogen intake. Increasing dietary N intake is associated with increased water intake and reduced plasma osmolality; to maintain plasma osmolality at optimum levels, the animal increases water excretion

in urine (Bannink et al., 2010). In this experiment, the dietary addition of DWP tended to increase N intake which may have indirectly increased water intake, leading to increased water excretion in urine. In addition, there was an increase in total urine N excretion (g/d) which also is known to increase urine volume (solute removal from the body requires a constant volume of water) (Bannink et al., 2010). Increasing dietary RDP content also tended to increase urine volume in this experiment. The increase in urine volume observed with increased dietary RDP content is likely due to the tendency for increased N intake. As explained above, increased N intake is associated with increased water intake and its subsequent excretion in urine.

Altering the proportion of RDP in the diet of lactating Holstein cows had no effect on urinary urea-N excretion, and total urinary N excretion (both g/d and as a proportion of N intake). It had been anticipated that increasing dietary RDP content would increase total urinary urea-N and urinary N excretion because increasing dietary RDP content is known to increase ruminal NH_3 concentration (Bailey et al., 2012; Mutsvangwa et al., 2016), and ruminal NH_3 is the source of the bulk of N used for hepatic urea synthesis (Storm et al., 2012). However, in this experiment ruminal NH_3 concentration did not respond to changes in dietary RDP content. Similar results to those observed in the present study were reported by Mutsvangwa et al. (2016), in an experiment that compared a low RDP diet (9.4 and 10.3% of dietary DM) with a high RDP diet (11.0 and 12.1% as a % of dietary DM), and no changes in urinary urea-N and urinary N excretion were observed. Savari et al. (2017) also saw no changes in urinary urea-N and urinary N excretion when dietary RDP intake was altered (10.0 vs 10.8 % RDP). Hristov et al. (2004), also investigated the effects of dietary RDP contents (9.4 vs 11.6% of dietary DM) in Holstein cows and reported that increasing dietary RDP content tended to increase urinary N loses; however, when urinary N losses were expressed as a proportion of N intake there was no difference in urinary N loss. However, in experiments with a larger difference in dietary RDP content between treatments, there is an observed change in urinary N losses. Gressley and Armentano (2007) fed either a diet with adequate RDP or a diet with 28% less RDP (i.e., 10.1% vs 7.4% RDP as % of dietary DM) which reduced total urinary N excretion (both g/d and % of N intake). Kalscheur et al. (2006) fed diets with increasing dietary content of RDP (6.8, 8.2, 9.6, and 11.0% of DM) to cattle, which linearly increased predicted urinary N excretion (calculated as MUN (mg/dL) $\times$ 12.54). These results seem to suggest that modest changes in ruminal RDP supply as in the present experiment have no major impact on urinary N excretion.

In this current study, increasing dietary RDP content had no effect on fecal N excretion on a quantitative basis but when fecal N was expressed as a proportion of N intake, fecal N excretion decreased from 33.9% to 31.6%. In agreement with these results, Hristov et al. (2005) altered the RDP ratio from 60:40 to 65:35 and reported that fecal N excretion on a quantitative basis was not affected; however, when fecal N excretion was expressed as a proportion of N intake, fecal N excretion was reduced. Similarly, Kalscheur et al. (2006) gradually increased dietary RDP content from 6.8 to 11% which linearly decreased predicted fecal N excretion. However, contrary to these experiments, Reynal and Broderick (2005) increased dietary RDP content from 10.6 to 13.2% of DM without affecting fecal N excretion. The reduction in fecal N excretion in the current experiment led to a decrease in total N excretion when expressed as a proportion of N intake from 64.1% to 61.6%. The reduction in fecal N and total N excretion is a result of increased total tract CP digestibility during this experiment which led to a reduction in fecal N excretion when expressed as a proportion of N intake when dietary RDP content was increased.

In the current experiment, partial replacement of barley grain with DWP increased urinary and fecal N excretion in absolute amounts; however, when expressed as a proportion of N intake there was no change in both fecal and urinary N excretion. The increase in fecal and urinary N excretion in absolute amounts is likely a result of the tendency for increased N intake when DWP was added to the diet, as both fecal and urinary N excretion are known to be responsive to N intake (Mutsvangwa et al., 2016). The expectation in the current experiment was that partial substitution of barley grain with DWP would decrease the proportion of intake N excreted in the urine. Increasing diet fermentability is known to reduce ruminal NH_3 concentration (Bailey et al., 2012; Chibisa et al., 2015), and since the bulk of N used for endogenous urea synthesis is derived from ruminal NH_3 (Storm et al., 2012), this would ultimately reduce endogenous urea synthesis and urinary urea-N excretion which constitutes the bulk of urinary N. However, in this experiment increasing diet fermentability (by substituting barley grain with DWP) had no effect on ruminal NH_3 concentration, urinary urea-N excretion (g/d or % of urinary -N), and UER to GIT. In past experiments, the effects of substitution of grain starch with simple sugars have been variable. Chibisa et al. (2015) partially replaced either barley or corn starch with DWP up to 6% DM, and there was no change in both urinary and fecal N excretion (g/d or % of N intake). In contrast to the current experiment and Chibisa et al. (2015), Broderick et al. (2008) observed a linear decrease in urinary N and urinary urea-N excretion when dietary starch was partially replaced by sucrose in

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dairy cows. However, when De Seram (2016) partially replaced barley grain with DWP up to 11.5% DM, there was a linear increase in fecal N excretion while quadratic response was observed in urinary-N excretion, with urinary N excretion initially increasing in the diet with 3.75 % DWP and subsequently decreasing and the diet with the highest DWP content excreting similar urinary N as the control diet (without added DWP). Despite the increases in urinary N and fecal N (g/d) in the current experiment, total excretion of N (both g/d and % of N intake) was not affected by the partial replacement of barley grain with DWP.

In this experiment all experimental diets had positive nitrogen balance, apparent nitrogen balance was similar in cows fed 9.5 and 11.5% RDP with added DWP, but apparent nitrogen balance was higher in cows fed high RDP compared to those fed low RDP without added DWP (interaction), and this same trend was observed with cow's productive N. These results suggest that the dietary addition of DWP increases nitrogen balance in the low RDP diet. The trend observed with apparent nitrogen balance and productive is due to differences in dietary CP content between the dietary treatment, and similar numerical trends were also observed with N intake.

5.6 Whole-body urea kinetics

As indicated elsewhere, the efficiency of N utilization in dairy cows is poor, so ruminant nutritionists continue to explore strategies to improve N efficiency. One such strategy focuses on reducing endogenous urea-N production and/or increasing the proportion of endogenously produced urea-N that is recycled to the GIT. Urea-N recycling is an evolutionary adaptation that allows ruminants to divert endogenous urea-N away from urinary excretion towards secretion into the GIT where it can potentially be used by ruminal microbes for microbial protein synthesis. Ruminal microbes washed out of the reticulorumen can potentially provide up to 75% of the metabolizable protein available to the animal in the small intestines (Dewhurst et al., 2000). In essence, urea recycling helps maintains a steady supply of N to the rumen, which helps maintain microbial growth in-between meals and when dietary N supply is insufficient to maintain microbial growth (Lapierre and Lobley, 2001). One of the major objectives of this experiment was to investigate the influence of DWP and RDP on whole body urea-N kinetics.

Increasing the dietary RDP content in this experiment from 9.5% to 11.5% of dietary DM reduced endogenous urea-N production by 14.4%. The expectation was that increasing the proportion of dietary RDP would increase endogenous urea production since dietary RDP content

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has a positive relationship with ruminal NH_3 concentration (Wickersham et al., 2008), and ruminally-derived NH_3-N is the main source of N for endogenous urea synthesis (Storm et al., 2012). However, in the literature, altering dietary RDP content seldom leads to changes in endogenous urea-N production (Kiran and Mutsvangwa, 2007; Bailey et al., 2012; Mutsvangwa et al., 2016), and UER is more responsive to nitrogen intake with a correlation of r = 0.96 (Huntington and Archibeque, 2000). In the current experiment, N intake tended to increase with increased dietary RDP content and there was a numerical increase in ruminal NH_3 concentration; however, this did not stimulate an increase in UER. It is not clear why UER decreased when N intake tended to increase and when there was a numerical increase in ruminal NH_3 concentration in this experiment.

Ruminants recycle between 40-80% of endogenously synthesized urea-N to the GIT, and the amount depends on the diet (Lapierre and Lobley, 2001). Altering dietary composition to increase the proportion of endogenous urea-N recycled to the GIT is one strategy which can be used to increase N efficiency and reduce urinary urea excretion. Endogenous urea-N recycled to the reticulorumen is hydrolyzed into NH_3 and CO_2 by microbial urease, and the liberated NH_3 can then potentially be used by ruminal microbes for anabolic purposes.

In the current experiment increasing the dietary RDP content by two percentage units numerically decreased the fractional transfer of UER to the GIT from 73% in the low RDP diet to 68% in the high RDP diet. Additionally, when expressed as g/d, GER tended to decrease with increasing dietary RDP content. Urea-N transportation from blood circulation into the GIT is by facilitated diffusion through urea transporter proteins (UT-B and UT-A) and aquaporins (AQP-3, 7, and 10) present in the GIT epithelia (Abdoun et al., 2006; Walpole et al., 2015). Endogenous urea-N production is positively correlated with the transfer of urea-N to the GIT (Archibeque et al., 2001; Marini et al., 2004; Recktenwald et al., 2014). In the current experiment, there was a decrease in UER, while GER only showed a numerical decrease. The reason why GER and UER trends were not similar is that this relationship is nonlinear, transfer of urea-N into the GIT is dependent on the blood-GIT concentration gradient maintained by microbial urease (urease converts urea into NH_3 and CO_2), and ruminal epithelial cell permeability. Microbial urease activity and GIT epithelial cell permeability is modulated by ruminal NH_3 concentration. Ruminal NH_3 concentration is negatively correlated with the activity of microbial urease, as urease helps

maintain a concentration gradient that facilitates diffusion of urea-N into the GIT by converting urea-N into NH_3 and CO_2, (Cheng and Wallace, 1979; Rémond et al., 1996). Ammonia also modulates urea transporter proteins, by altering luminal pH of epithelial cells, increased NH_3 increases luminal pH, which in turn reduces the permeability of the epithelial wall to urea-N (Lu et al., 2014). Additionally, NH_3 transport across the GIT epithelia also utilizes UT-B, thereby competing with urea-N for transport chains (Lu et al., 2014). In this experiment, there was a numerical trend towards increased ruminal NH_3 concentration with increased dietary RDP content, leading to the reduced transfer of urea-N (both g/d and as a proportion of UER) into the rumen.

Endogenously synthesized urea-N is recycled to all the portions of the GIT; however, only urea-N transferred to the reticulorumen can potentially contribute to anabolism as microbial protein that is synthesized from urea-N recycled to hindgut is excreted in feces because it cannot be digested or absorbed (Lapierre and Lobley, 2001; Davies et al., 2013). Urea-N utilized for anabolism is calculated as the difference between urea-N transferred to the gut minus endogenous urea-N excreted in feces and urea-N returned to the ornithine cycle (Lobley et al., 2000). According to Lobley et al. (2000), UUA is mainly composed of ruminal microbial protein synthesized from recycled-N.

Increasing the dietary content of RDP also did not affect UUA or the proportion of GER used for UUA in the current experiment. The reason why the anabolic use of recycled N was not affected by changes in dietary RDP content in the current experiment is likely that the diet supplied adequate N for ruminal microbes, and the limit for anabolic use of N may have been reached as suggested by Lobley et al. (2000). Lobley et al. (2000) suggested that the limiting factor for microbial use of N may be ruminal availability of energy. According to Schwab et al. (2005), the minimum ruminal concentration needed to maintain optimum microbial growth is 5 mg/dL (3.6 mmol/L), and dietary treatments in this experiment produced ruminal concentrations above this threshold (12.5 mmol/L in the low RDP diet and 14.6 mmol/L in the high RDP diet). Additionally, all diets met the minimum RDP levels needed to maintain microbial function (NRC, 2001). The results observed in this experiment mirror observations reported in other experiments that have investigated the effects of altering dietary RDP contents on UUA (Bailey et al., 2012; Davies et al., 2013; Mutsvangwa et al., 2016).

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In this experiment, endogenous urea-N excretion in feces showed a 13.5% decrease with increased dietary RDP content, while the proportion of UER excreted in feces did not change. In contrast to the current experiment, Bailey et al. (2012) ruminally dosed steers either 120 g/d or 240 g/d of casein (a source of RDP), which increased UFE. Similarly, Wickersham et al. (2008) ruminally dosed steers with casein, and observed that increasing the casein dose from 0 to 177 mg of N/kg of BW linearly increased UFE. The main factor influencing urea-N excretion in feces is the supply of fermentable carbohydrates to the hindgut (Thornton et al., 1970; Oncuer et al., 1990). In the current experiment, the supply of fermentable carbohydrates to the hindgut was not measured; however, the diets were not designed to increase the supply of fermentable carbohydrates to the hindgut. The most probable reason why UFE decreased is that it was a direct response to the 13.2% decrease in UER since the fractional transfer of GER to feces did not change. Urea-N transfer across the GIT is by facilitated diffusion, thus increasing UER would increase the steepness of the diffusion gradient thereby potentially increasing the transfer of urea-N into the GIT.

Partial substitution of barley grain with DWP up to 11.2% of dietary DM increased endogenous urea-N synthesis by 11%. The expectation was that increasing dietary fermentation rate by substituting starch (in the form of barley grain) with lactose (in the form of DWP) would reduce UER. Increasing diet fermentation is known to increase microbial utilization of ruminal NH_3 (Bailey et al., 2012; Chibisa et al., 2015), and ruminally-derived NH_3-N is the main source of N for endogenous urea synthesis (Storm et al., 2012). Dietary treatments in this experiment did not affect ruminal NH_3 concentration, and it is unlikely that the changes in UER were a result of ruminal NH_3 changes. However, it is important to realize that ruminal NH_3 concentration does not always equate to ruminal NH_3 production. Another factor that has an impact on endogenous urea production is N intake, with N intake being positively correlated with hepatic synthesis of urea (Mutsvangwa et al., 2016). In the current experiment, there was a tendency for increased N intake when DWP partially replaced barley grain. Furthermore, the amount of UER produced was almost equal to digestible N intake in both treatments. The UER to digestible N intake was 1.03 and 0.97 for the diet with and without added DWP respectively, proving that the change in UER was a direct result of increased N intake as opposed to DWP inclusion. In support of this conclusion, a similar experiment by De Seram (2016) observed a quadratic increase in UER with increasing DWP

inclusion, and the conclusion in that experiment was that the increase in UER was due to the observed the numerical increase in N intake with increased DWP inclusion rates.

Increased diet fermentability is known to increase the proportion of endogenous urea-N transferred into the GIT (Kennedy and Milligan, 1980; Huntington, 1989; Rémond et al., 1996). In the current experiment, increasing diet fermentability by substituting barley grain with DWP had no effect on GER or the proportion of UER transferred to the GUT. These results are in contrast to past experiments that have investigated the effect of increased diet fermentability by either substituting dietary grain with simple sugars or through grain processing. Gozho et al. (2008) increased barley's ruminal fermentability by pelleting, which increased GER. While Theurer et al. (1999) increasing starch fermentation rates by steam flacking corn, compared to steam or dry-rolled corn which also increased GER. Also in an experiment by De Seram (2016), where barley grain was partially replaced by 12.5% DWP, GER increased in a quadratic manner.

It has been suggested the increased transfer of urea-N into the rumen when diet fermentability is increased is due to increased epithelial cell permeability stimulated by SCFA (Abdoun et al., 2006, 2010). Increasing butyrate concentration has been shown in experiments by Engelhardt et al. (1978) to increase urea transport. This increase is likely mediated by increased expression of urea transport protein across the rumen epithelial wall. Studies with steers fed either forage or concentrate diets have shown that increasing diet fermentability with concentrates increased expression of UT-B (Simmons et al., 2009) and AQP-3 mRNA in ruminal epithelia (Walpole et al., 2015). Apart from the increased expression of transport channels, another mechanism may involve increased ruminal epithelial surface area for absorption in response to increased SCFA (butyrate in particular) (Penner et al., 2014). Besides SCFA, ruminal NH_3 also regulates urea transport across the GIT epithelia. Ruminal NH_3 inhibits urea recycling by inhibiting urease (creating an unfavourable concentration gradient) (Cheng and Wallace, 1979; Rémond et al., 1996), lowering epithelial cell pH (reducing cell permeability) and directly competing with urea for use of UT-B with urea (Lu et al., 2014). In the current study, dietary addition of DWP had no impact on ruminal NH_3 concentration and furthermore butyrate concentration and total SCFA concentration decreased which may partly explain the lack of response of GER to partial replacement of barley grain with DWP in this experiment.

Endogenous urea-N recycled back to the GIT is hydrolyzed into NH_3 by microbial urease (Cheng and Wallace, 1979; Rémond et al., 1996), and is potentially available for microbial protein synthesis. Microbial protein synthesis and growth is energy-dependent and increasing dietary fermentable energy supply is known to increase NH_3 use by ruminal microbes in the synthesis of microbial protein (Nocek and Tamminga, 1991). Simple sugars have in the past been observed to increase MPS (Kim et al., 1999; Bailey et al., 2012), and increase the duodenal flow of microbial non-ammonia nitrogen (De Seram, 2016). According to the Cornell Net Carbohydrate and Protein System, sugar-utilizing bacteria produce 18% more microbial protein (NRC, 1996).

Based on this, the expectation was that increasing diet fermentability by replacing barley grain with DWP would increase utilization of recycled urea-N for anabolism. However, partial replacement of grain starch with DWP had no effect on UUA or the proportion of GER used for UUA. De Seram (2016) observed similar results when barley grain was partially substituted with DWP up to 11.5% in dairy cows. These results seem to support the suggestion that the benefits of increased diet fermentability by addition of simple sugars is buffered by the synthesis of storage carbohydrates by microbes from simple sugars and the resultant increased energy spilling. In this experiment, glycogen synthesis was not measured, but it has been demonstrated that in the presence of simple sugars, ruminal bacteria and protozoa (Lou et al., 1997; Hall, 2011) increase glycogen synthesis. As discussed elsewhere, glycogen synthesis is an energy consuming process and contributes to energy spilling (Lou et al., 1997b; Hall, 2011). The storage of simple sugars as glycogen in ruminal bacteria and protozoa shields those simple sugars from ruminal fermentation. This idea that ruminal bacteria and protozoa shield simple sugars from ruminal fermentation is further supported by the reduction in SCFA concentration and lack of effect on NH_3 concentration observed in the current experiment.

6   GENERAL DISCUSSION

Nitrogen utilization is low in dairy cows, up to 80% of intake protein is excreted in feces and urine (Arriola Apelo et al., 2014), leading to environmental degradation and corrosion of profits. Nitrogen utilization is low because of a mismatch between N and energy availability (Mccormick et al., 2001). Simple sugars have in the past been demonstrated to improve N efficiency by increasing MPS (Kim et al., 1999) and reducing urinary urea excretion (Broderick et al., 2008). However, there is a concern that increasing diet fermentability using simple sugars can increase animals' susceptibility to ruminal acidosis. There have been experiments that have demonstrated that dietary addition of simple sugars up to 12.5% TS does not increase the risk of ruminal acidosis (De Seram, 2016). Additionally, simple sugars have been shown to increase DMI (Penner and Oba, 2009), and DM digestibility (Chibisa et al., 2015). However, there is limited research on partial replacement of barley grain, which is more common in Western Canada, and there have been no studies to investigate the interaction between simple sugars and dietary RDP content. This experiment sought to fill in this knowledge gap.

Past studies have shown that simple sugars can be added to diets up to 13% DM without negatively affecting animal productivity or reducing ruminal pH (DeFrain et al., 2004; De Seram, 2016). In this current experiment dietary addition of 12.5% DWP DM (10.7% sugar content), did not affect mean, and minimum ruminal pH, or the time and area spent under the pH threshold of 5.8. Apart from upregulation of SCFA transport out of rumen that was demonstrated by an experiment by Chibisa et al. (2015), another factor that helps stabiles ruminal pH when more simple sugars are added to the diet is microbial glycogen accumulation. Ruminal microbes synthesize glycogen when carbohydrate availability exceeds microbial needs (Strobel and Russell, 1986) and this behavior is more pronounced in the presence of simple sugars (Lou et al., 1997b; Hall, 2011). Glycogen synthesis shields simple sugars from fermentation and reduces the rapid accumulation of SCFA characteristic of ruminal acidosis. Increased glycogen synthesis together with upregulation of SCFA transport out of the rumen may explain the lack of change in ruminal pH measurements in this experiment. This suggestion is supported by the lack of changes in ruminal characteristics that are usually associated with increased dietary fermentation rates like reduction in ruminal NH_3 concentration and increased SCFA concentration. In this experiment, there was no change in ruminal NH_3 concentration, and SCFA concentration decreased.

Partial replacement of barley grain with DWP in this experiment tended to increase DMI and OM digestibility. Since the diets contained similar NE_L (avg 1.51 ± 0.02 Mcal/kg of DM), increased DMI and OM digestibility should have increased milk yield; however, milk yield did not change. It is no clear why dietary addition of DWP did not change milk yield in this experiment. The potential benefits (increased milk yield) which are associated with increased diet fermentation may have been negated by increased energy spilling associated with increased sugar availability in the diet. In this experiment, energy spilling was not measured; however experiments by Lou et al. (1997a) and Hall (2011) demonstrated that in the presence of simple sugars, ruminal microbes increases glycogen synthesis. Glycogen synthesis is an energy consuming process that contributes to energy spilling, thereby diverting energy that would have been used for production.

Dietary addition of DWP had no significant effect on N balance in this experiment. Urinary N and fecal N excretion when expressed as g/d increased, however when expressed as a proportion of N intake there was no change. This increase was likely a result of the tendency for increased N intake associated with increased DWP content rather than an increase in actual DWP. Nitrogen intake is positively related to urinary N excretion in ruminants (Mutsvangwa et al., 2016). The reason why a partial substitution of barley grain with DWP did not affect N excretion is that NH_3 concentration and total-tract CP digestibility were not changed by DWP inclusion.

Endogenous urea-N increased with DWP inclusion in this experiment, however these results are most likely a result of digestible N intake instead of DWP intake. Nitrogen intake is correlated with endogenous urea-N synthesis (Mutsvangwa et al., 2016). Endogenous urea-N synthesis was similar to digestible N intake in this experiment, with a DN to UER ratio of 0.99 and 1.03 for the diet without and with added DWP respectively (UER 513.8 and 570.1 g/d, DN 517.9 and 552.0 g/d). Dietary addition of DWP did not affect the movement of urea-N in the body; this is so because DWP inclusion did not stimulate changes in ruminal NH_3 concentration. Ruminal NH_3 concentration has an impact on urea-N recycling back to the rumen and utilization by ruminal microbes (Lapierre and Lobley, 2001).

The anticipation in this experiment was that increasing dietary RDP content would increase DMI and cow productivity; however, DMI, milk yield and composition was not affected by dietary RDP change. Improvements often seen with increased dietary RDP content is because of increased N availability in the rumen, which increases microbial activity, feed digestibility, DMI and post-

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ruminal nutrient availability. In this experiment, there was a numerical increase in ruminal NH_3 concentration (12.5 vs 14.6mmol/L, $P = 0.12$), however this increase did not stimulate DMI, DM digestibility and overall animal productivity. Schwab et al. (2005) recommends a minimum ruminal NH_3 concentration of 3.6mmol/L to maintain ruminal microbial activity; in this experiment both treatments provided more than 3.6mmol/L NH_3 concentration. The ruminal ammonia concentration increase did not change DMI or productivity because both diets were not deficient in RDP.

The dietary RDP content used in this experiment are based on the NRC (2001)'s recommendations; the 9.5% RDP inclusion level is considered borderline deficient in terms of NRC (2001) recommendations, and the 11.5% RDP inclusion level is considered adequate. It has been suggested that the NRC (2001) overstates dietary RDP recommendations (Cyriac et al., 2008; Huhtanen and Hristov, 2009; Agle et al., 2010; Chibisa, 2013), and 8.8% is adequate to maintain animal productivity. Huhtanen and Hristov (2009) suggests that the reason why the NRC overstates RDP requirement is because it does not consider the contribution of recycled urea-N to ruminal NH_3 pool; in this experiment endogenous urea recycling contributed 48% as much N as intake N to the rumen N pool (382.7 g/d GER vs. 794.5g/d intake N).

In this experiment increasing dietary RDP content had no effect urinary urea-N, while fecal N (% of N intake) and overall N excretion (% of N intake) decreased in the high RDP diet. It had been expected that the low RDP diet would decrease urinary urea-N and total urinary N excretion. Unlike fecal N, urinary N is high in urea which easily vaporizes into NH_3, contributing to environmental pollution (Arriola Apelo et al., 2014), it is important to reduce urinary N excretion. Increasing dietary RDP content is known to increase ruminal NH_3 concentration and ruminal NH_3 is main source of N for endogenous urea synthesis. However, in this experiment there was only a numerical increase in ruminal NH_3 (12.5 vs 14.6 mmol/L, $P = 0.12$), this change may not have been large enough to stimulate urea-N excretion. Even though ruminal NH_3 concentration increased UER decreased; it is not clear why UER decreased in this experiment.

7   CONCLUSIONS

Partial substitution of barley grain with DWP up to 11.2% to achieve a dietary sugar content of 10.7% did not increase the susceptibility of cows to ruminal acidosis and had no impact on animal productivity. Dietary addition of DWP had no significant impact on N utilization or urea recycling. Increasing dietary RDP content reduced overall N excretion in this experiment; however, the dietary RDP changes in this experiment were likely not large enough to stimulate changes in animal productivity or significantly change urea-N recycling. There was no meaningful dietary interaction between partial substation of barley grain with DWP and dietary RDP content.

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